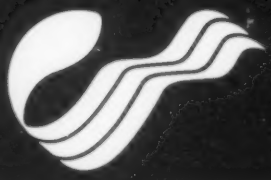

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Journal canadien des sciences halieutiques et aquatiques

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Multiple-Frequency Acoustic Backscattering and Zooplankton Aggregations in the Inner Scotian Shelf Basins

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Acoustic backscatter observations at 12, 50, and 200 kHz in the inner Scotian Shelf basins reveal large populations of silver hake (*Merluccius bilinearis*) and its principal prey, the euphausiid *Meganyctiphanes norvegica*. Multichannel sonar colour imagery facilitates separation of fish from euphausiids and delineation of their separate diurnal migration patterns. Silver hake acoustic abundances are consistent with midwater trawl sampling. Acoustic column densities for mature *M. norvegica* of approximately 1000/m^2 exceed net sampling column densities by over 1 order of magnitude indicating strong net avoidance. Numerical simulation of euphausiid backscattering using a fluid cylinder model and natural distribution of scatterer orientations yields an approximate 20 log L target strength length dependence and little frequency domain dependence in the "geometric" scattering regime. Quantitative interpretation of euphausiid scattering in situ must account for randomness in their orientations.

Des données sur la rétrodiffusion acoustique à 12, 50 et 200 kHz recueillies dans les bassins internes de la plateforme Scotian ont révélé la présence d'importantes populations de merlu argenté (*Merluccius bilinearis*) et de l'euphausiacé *Meganyctiphanes norvegica*, sa principale proie. L'imagerie couleur générée par un sonar multicanal facilite la séparation des poissons et des euphausiacés et la délimitation de leur régime de migration diurne. L'abondance du merlu argenté obtenue par relevés acoustiques est compatible avec les données obtenues par échantillonnage au chalut mésopélagique. Les densités d'individus matures de *M. norvegica* dans la colonne acoustique, soit environ 1 000/m^2, sont supérieures aux densités d'échantillonnage au filet par plus d'un nombre de grandeur; ceci indique un taux élevé d'évitement du filet. Une simulation numérique de la rétrodiffusion des euphausiacés basée sur un modèle d'un cylindre de fluide et la distribution naturelle des orientations des cibles donne un rapport approximatif entre la longueur et l'index de réflexion de 20 log L; le domaine de fréquence influe peu en régime "géométrique" de diffusion. L'interprétation des valeurs de diffusion des euphausiacés in situ doit tenir compte du caractère aléatoire de leurs orientations.

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The deep basins (>200 m depth) of the Nova Scotia Shelf contain particularly high concentrations of Crustacea supporting an extensive fish stock biomass. Previous studies in Emerald Basin (Sameoto and Herman 1990) have revealed large populations of the copepods (*Calanus finmarchicus*, *C. glacialis*, and *C. hyperboreus* and of the euphausiid *Meganyctiphanes norvegica*. The copepods concentrate below 200 m during the summer to winter periods. The euphausiids are found at the same depths as the *Calanus* copepods during daylight hours but vertically migrate to the upper 30 m at night. The copepods do not migrate. The fish community is dominated by silver hake (*Merluccius bilinearis*) (Waldron 1988; Koeller et al. 1990) which diurnally migrates.

The spatial–temporal distributions and interrelationships of a mixed population, ranging in size from fish to zooplankton, can be studied more readily by acoustic echosounding at several frequencies than by direct net sampling. However, net data are necessary to define the principal organisms responsible for the scattering and to enable more precise abundance estimates from acoustics using theoretical and empirical scattering models. In Emerald Basin and in the adjacent LaHave Basin, a variety of nets and other in situ sampling gear were used simultaneously with multifrequency acoustic backscatter recording. The objectives of this study were to (1) demonstrate the use of acoustics to separate different biological populations, (2) delineate population behaviour and interactions at high temporal and spatial resolution, and (3) quantitatively assess populations using acoustics both alone and in conjunction with net sampling. The last objective requires numerical assessment of the effects of zooplankton orientation using theoretical scattering models.

TABLE 1. Transducer parameters.

Freq. (kHz)	Type	Pattern	Beam width (−3 dB)
12	EDO 323B	Circular	35°
50	FURUNO 50B-12	Rectangular	18°
200	FURUNO 200B-8	Circular	5.4°

Methods

Database

We utilized a June 1988 acoustic database for which concurrent plankton net sampling and fish trawl databases were also available. Video observations using a HYSUB 5000 ROV in Emerald Basin during June 1989 are mentioned in the discussion.

Acoustic Instrumentation

Acoustic data were gathered on three frequencies simultaneously using modified DATASONICS DFT-210 transceivers with digital time variable gain (TVG). Downward-directed sonar transducers operating at 50 and 200 kHz (Table 1) were towed at 6–7 m depth below the surface bubble layer (Novarini and Bruno 1982). A third 12-kHz transducer was hull mounted. Acoustic calibration at 50 and 200 kHz was by normal water surface reflection in an enclosed pool. The 12-kHz hull transducer was calibrated in situ using a reference ping-pong ball target lowered within the acoustic far field (Welsby and Hudson 1972).

Biological Sampling

Mesozooplankton samples were taken with the Bedford Institute of Oceanography Net and Environmental Sensing System (BIONESS) (Sameoto et al. 1980). This was an opening and closing net sampler with 10 nets with a mouth opening of 1 m^2, each with a mesh size of 243 μm. The BIONESS continuously measured temperature and salinity during tows by means of a GUILDLINE INSTRUMENTS digital CTD. Flow through the nets was monitored with external and internal flowmeters. The BIONESS was towed at 1.5 m/s while it was slowly lowered along an oblique path to the desired depths where the nets were opened and closed on command from the ship. Samples were taken within each 5- to 10-m depth stratum from the surface to the thermocline, and at approximately 10-m intervals below the thermocline. Each net filtered between 30 and 150 m^3 of water.

An in situ optical zooplankton counter (Herman 1988) was mounted on the BIONESS to both count and size zooplankton of 0.2–30 mm equivalent circular cross-sectional diameter. An optical zooplankton counter was also mounted on a BATFISH vehicle (Herman 1988; Herman et al. 1991), an undulating deep-towed profiler, to provide horizontal and vertical profiles of the sizes and concentrations of zooplankton from the surface to within 25 m of the bottom.

Pelagic fish were sampled with a midwater International Young Gadid Pelagic Trawl (IYGPT) (Potter et al. 1990) with a mouth opening of 100 m^2 and a cod-end mesh size of 5-mm stretch mesh. Trawl tow depths were selected by reference to acoustics. During midwater trawling the two sampling vessels ran 0.2–0.5 km apart on parallel courses; one ship trawled while the other gathered acoustic data and sampled with the BIONESS. Tow distances were usually in the 3- to 3.5-km range.

TABLE 2. Sonar operational parameters for 1988 database.

Freq. (kHz)	Pulse (ms)	Power (kW)	Receiver B.W. (kHz)	TVG range (m)	Absorp. Coeff. (dB/km)
12	2	0.4	1	5–500	1.1
50	2	0.4	1	5–500	16.1
200	5	2	1	2–200[a]	66.6

[a]TVG depth range extended by digital postprocessing.

Acoustic Sampling Procedures

Recording of multichannel acoustic backscattering was normally initiated simultaneously with the opening of the first BIONESS net dedicated to narrow vertical interval sampling. Recording continued throughout the net sampling sequence. Typically about 1000 consecutive acoustic transmissions or "pings" were logged at a transmission rate of 1 ping/s while the BIONESS sampler and acoustic fish were towed at 1.5 m/s or occasionally 2.5 m/s. The detected signal envelopes from the three transceivers were each digitized at a 5-kHz rate and the resultant data stream collected by a DEC MicroVAX II computer, time-stamped, and logged in total to digital tape for later detailed analysis. Near the end of an acoustic recording session an additional set of 300 acoustic "pings" was recorded with the transmitter output suppressed. The resultant receiver outputs established the receiver-generated plus ambient noise level which was used in subsequent data reduction. Acoustic recording was also conducted concurrent with BATFISH tows at a rate of 1 ping/5 s or 1 ping/10 s while towing at 4.5 m/s. Additional acoustic operational parameters appear in Table 2.

Acoustic Data Reduction

Digitally acquired acoustic data were displayed on a DEC VAX 750 based PERCEPTRON image processing system to assess data quality, to delineate major features, and to select sections for quantitative analysis. Colour composite sonar sections were produced by additively superimposing two or three echograms at different frequencies, each echogram represented by shades of a separate primary colour with higher intensities corresponding to higher backscattering levels. We used red, green, and blue for the 12-, 50-, and 200-kHz channels, respectively. This display technique is analogous to the NASA LANDSAT thematic mapper false colour imagery. Since the frequency dependence of acoustic backscattering is intimately related to organism size, colour composite sections should depict aggregations of organisms of grossly differing physical size in contrasting colours. Large scatters, such as fish, scatter efficiently over a wide frequency range extending down to 12 kHz. Centimetre-size zooplankton are only detected at 50 and 200 kHz.

For quantitative study, acoustic sections were reduced to profiles of volume backscattering strength, S_v, versus depth (Clay and Medwin 1977). Profile data smoothing consistent with preservation of total acoustic signal power was accomplished by squaring and averaging signal amplitudes over eight samples (1.6 ms) in the vertical and 300 pings (5 min) along the transect or section. Smoothed signal power estimates were corrected for unwanted receiver plus ambient noise power by subtracting a similarly averaged receive-only noise sample. Signal power to S_v conversion was accomplished by utilizing integrated beam width factors (Clay and Medwin 1977) appropriate to baffled circular piston transducers at 12 and 200 kHz and by utilizing

a numerically derived factor for the 50-kHz rectangular transducer computed from measured beam patterns. Displayed S_v versus depth profiles used fixed nominal sounder TVG absorption corrections (Table 2) and an acoustic velocity (c) of 1485 m/s. All profile depths are accurate to within 3 m using the above value of c.

For acoustic modelling, volume backscattering strength (S_v) profiles were further corrected for precise acoustic path absorption versus depth using measured temperatures and salinities and the methodology of Clay and Medwin (1977) which included shear and bulk viscosity dependencies on temperature and depth. Direct interchannel comparisons of profile S_v levels should be restricted to cases of diffuse layers with thicknesses greater than the vertical resolution imposed by differing wave front curvatures (beam widths) and pulse lengths. For example, wave front curvature (between -3 dB points) at 200 m range would produce vertical smearing of about 9, 2.5, and 0.2 m at 12, 50, and 200 kHz, respectively, using the beam widths listed in Table 1. The relatively long transmit pulse lengths used (Table 2), necessary to achieve acceptable signal to noise ratios from scattering layers at long range, superimpose an additional vertical smearing of about 3.8 m at 200 kHz, and 1.5 m at both 50 and 12 kHz.

Echo integration is a superior technique for effecting interchannel volume backscattering strength intercomparisons when differing beam widths or pulse lengths are utilized and when the scattering layers are also thin or highly structured. The volume backscattering coefficient, the nonlogarithmic form of volume backscattering strength (Clay and Medwin 1977), is computed and vertically integrated completely through the feature of interest. Reversion to logarithmic form after integration and comparison between channels yields the "effective" S_v difference.

Modelling of Acoustic Backscatter

For a population of identical scatterers, volume backscattering strengths can be related to organism number density (number per cubic metre) and organism target strength (TS):

$$10 \log_{10} (\text{Number density}) = S_v\,(\text{dB}) - \text{TS(dB)}. \quad (1)$$

Fish target strength dependencies on size and acoustic frequency have traditionally been expressed by empirical formulae. Use will be made of the Forbes (1975) formulae for cod, haddock, and pollack in an arithmetically averaged form:

$$\text{TS} = 23.9 \log_{10} L - 3.9 \log_{10} \lambda - 31.6 \text{ dB (MKS units)} \quad (2)$$

where TS is measured in dorsal aspect, and fish length $L > \lambda$, where λ is the acoustic wavelength.

For modelling of zooplankton acoustic scattering, considerable uncertainty still exists as to the target strengths of real organisms in situ. Our philosophy was to use the most realistic models presently available together with the best observations of orientational behaviours and physical parameters. At the current state of our knowledge, quantitative results so derived should be considered very approximate. Theoretical scattering models have been developed for simple geometric forms such as fluid spheres and cylinders embedded in a contrasting fluid medium (Anderson 1950; Johnson 1977; Greenlaw 1977; Stanton 1987, 1989). We use the "high-pass" straight fluid cylinder model and "high-pass" fluid sphere model of Stanton (1989) assuming that modal resonances predicted by rigorous solutions are, in reality, smoothed out by nonideal scatterer shapes and variability in scatterer size. Specific mathematical formulations appear in Table 3.

Many zooplankton are elongate organisms which can be roughly modeled as fluid cylinders. Photographic studies of specific zooplankton have shown a high degree of randomness in their (length axes) orientations to the horizontal. Orientation must be properly modelled for useful quantitative interpretation.

We compute an "effective" target strength from the average backscattering cross-section of an ensemble of identical cylinders oriented either completely randomly in two-dimensional (2-D) space (the projections of cylinder axes displaying no systematic pattern on the unit enclosing sphere) or with axes orientations to the horizontal plane conforming to a defined distribution function. Specifically we have assumed a Gaussian angular probability density function (PDF) symmetric about the horizontal orientation of the form

$$P(\theta)_{\text{2-D}} = \frac{1}{\sigma(2\pi)^{1/2}} e^{-(\theta^2/2\sigma^2)} \quad (3)$$

where σ is the standard deviation of the angular distribution. The 2-D distribution function is of a type readily obtained photographically. Photographic observations are generally restricted to a vertically oriented thin plane of focus. Orientational statistics are derived for selected organisms orientated close to this plane. For computation of an ensemble average, the 2-D planar PDF of axes orientations to a horizontal vector must first be converted into the three-dimensional (3-D) volume PDF of axes orientations to a horizontal plane:

$$P(\theta)_{\text{3-D}} \propto P(\theta)_{\text{2-D}} \cdot \cos\theta. \quad (4)$$

Note that the 2-D and 3-D PDF's are similar for moderate values of θ but diverge for large θ. If the 2-D PDF is completely random (i.e. independent of θ), than the 3-D PDF is $\alpha \cos\theta$.

Starting with the 2-D PDF, ensemble averaging with renormalization proceeds:

$$\tilde{\sigma}_{bs} = \frac{\int_0^{\pi/2} \sigma_{bs}(\theta) \cdot P(\theta)_{\text{2-D}} \cdot \cos(\theta)\, d\theta}{\int_0^{\pi/2} P(\theta)_{\text{2-D}} \cdot \cos(\theta)\, d\theta}. \quad (5)$$

In practice the integrals are evaluated numerically permitting arbitrary choice of the distribution functions:

$$\text{TS (effective)} = 10 \log \tilde{\sigma}_{bs}. \quad (6)$$

In applying relation (5), one should be aware that the Stanton (1989) fluid cylinder model is considered accurate only for small θ. For the organism sizes and acoustic frequencies encountered in our applications the $\sigma_{bs}(\theta)$ term is heavily weighted toward smaller θ values.

Inverse Modelling

For a population of identical organisms the difference between S_v levels observed at two acoustic frequencies defines zooplankton size. The Stanton high-pass fluid sphere model was solved algebraically in this inverse manner. On assigning realistic values of g and h, using two observational frequencies

TABLE 3. Scattering model formulations from Stanton (1989).

$$TS = 10 \log (\sigma_{bs})$$

where for high-pass fluid cylinder:

$$\sigma_{bs} = \frac{\frac{1}{4} L^2 (Ka)^4 \alpha_{\pi c}^2 s^2 G}{1 + [\pi (Ka)^3 \alpha_{\pi c}^2]/(R^2 F)}$$

and for high-pass fluid sphere:

$$\sigma_{bs} = \frac{a^2 (ka)^2 \alpha_{\pi s} G}{1 + [4 (ka)^4 \alpha_{\pi s}^2]/(R^2 F)}$$

where:

σ_{bs} is backscattering cross-section
L is cylinder length
a is radius of either cylinder or of sphere in respective model
k is acoustic wave number, $2\pi/\lambda$, where λ is acoustic wavelength
K $= k \sin \theta$, where θ is the angle of cylinder ensonification measured from cylinder axis
s $= \sin (kL \cos \theta)/kL \cos \theta$, the directivity function for the fluid cylinder

$$\alpha_{\pi c} = \frac{1 - gh^2}{2gh^2} + \frac{1 - g}{1 + g}$$

$$\alpha_{\pi s} = \frac{1 - gh^2}{3gh^2} + \frac{1 - g}{1 + 2g}$$

$$R = \frac{gh - 1}{gh + 1}$$

where

R is Raleigh reflection coefficient of body in seawater
g is ratio of body density to density of seawater
h is ratio of body compressional wave acoustic velocity to corresponding value for seawater
F heuristic function to account for acoustic losses in body
G heuristic function to account for residual modal resonances

Note: We use the simplifying approximation $F = G = 1$ for all calculations

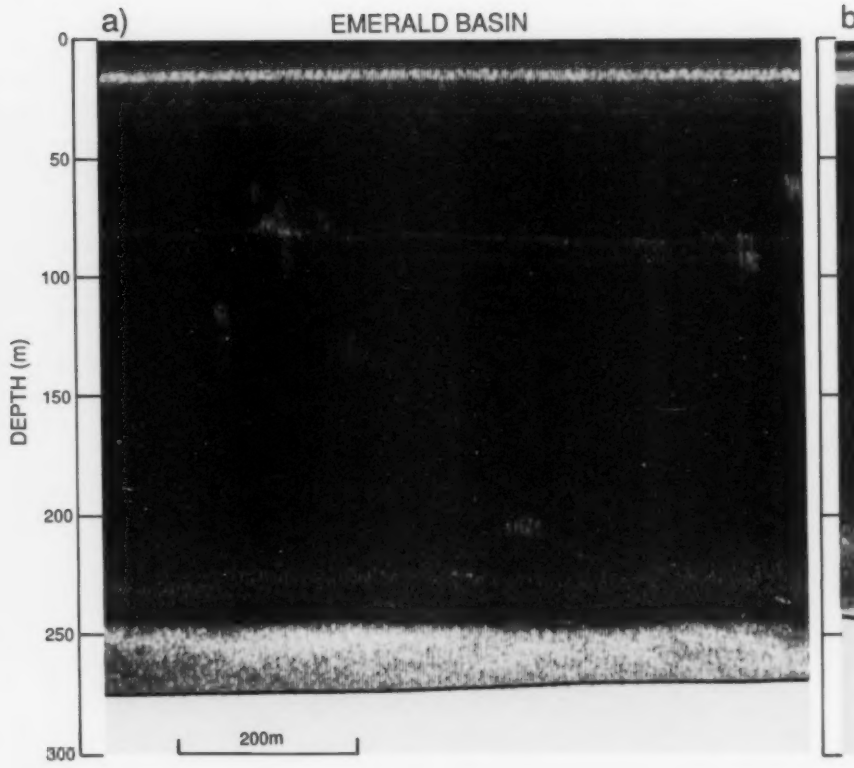
spanning the Raleigh to geometric transition region, and further assuming $G = F = 1$, the fluid sphere radius was directly obtained (more complex functional dependencies of G and F may introduce nonunique solutions requiring more observational frequencies to resolve). Once size was determined, target strength was also defined and concentration (number density) followed from relation (1).

Inverse modelling was also possible with the high-pass cylinder model. One proceeded by introducing an additional empirical relation between cylinder radius and length appropriate to the anticipated biological type. We utilized empirical relations between zooplankton length and volume developed by Greenlaw and Johnson (1982). On setting cylinder length equal to zooplankton body length, the Greenlaw and Johnson relationship was used to determine a cylinder radius consistent with the empirical zooplankton volume. Cylinder parameters were extracted from observations at two frequencies by a successive approximation numerical search algorithm in which organism volume, or equivalently the organism equivalent spherical radius (e.s.r.), was progressively incremented through the range of interest. Again assuming $F = G = 1$, unique solutions using only two appropriately chosen frequencies were possible for fixed ensonification angles close to broadside as well as for Gaussian and completely random oriented ensembles. More specific computational details and consideration of complicating effects such as finite transducer beam width are treated below.

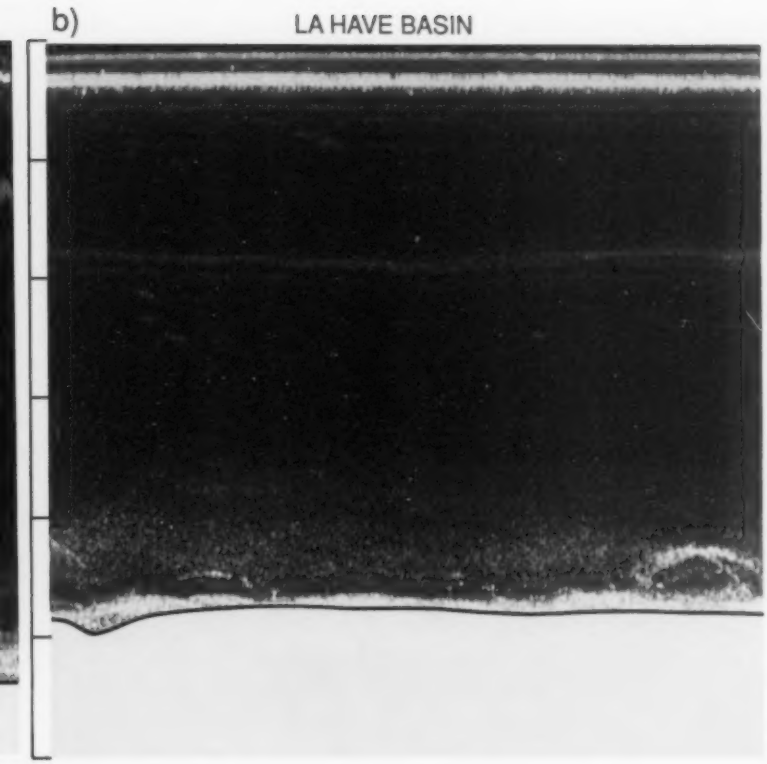
Results

Basin Echograms (Daytime)

Colour composite echograms for 0.8-km transects in Emerald and LaHave basins show rather similar scattering patterns in both basins (Fig. 1). We draw attention to several less obvious details and interpretational considerations. For clarity, red levels have been enhanced so that scattering horizons reflecting approximately equally well at all three frequencies appear distinctly red. The near-bottom "red" layers reproduce in places as white due to the limited dynamic range of the imaging and subsequent photographic processes. These "red" layers have sharply defined upper boundaries and, in Emerald Basin, the layer decreases in intensity toward bottom. A 10- to 15-m-thick, low-reflectivity (dark) gap is observed between the top of the "red" layer and the base of the overlying "blue" layers in both basins. Other multiple, semicontinuous red layers between 80 and 100 m appear no thicker than the 1.5-m vertical resolution imposed by the 2-ms, 12-kHz pulse width. Additional fainter red bands above 40 m are artifacts of hull-mounted transducer reverberation as evident from their spatial



1. Multifrequency acoustic sections from Scotian Shelf basins reproduced in superimposed additive
intensities scaled to maximize discrimination. Each section is of 512 s duration. (a) Central Emerald
June 11, 1988; (b) central LaHave Basin at nominal location 43°43.2′N, 63°45.7′W commencing at



dditive primary colours red (12 kHz), green (50 kHz), and blue (200 kHz) with relative
Emerald Basin at nominal location 43°44.8′N, 63°02.0′W commencing at 1455 ADT,
ncing at 1531 ADT, June 12, 1988.

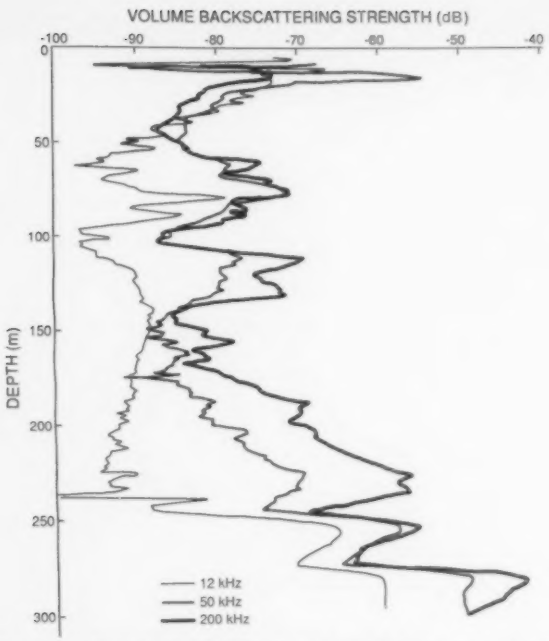


FIG. 2. Profiles of volume backscattering strength (S_v) versus depth at 12, 50, and 200 kHz in central Emerald Basin. Analysed data comprises first 300 s (300 pings) of profile shown in Fig. 1a. Bottom occurs at about 270 m depth.

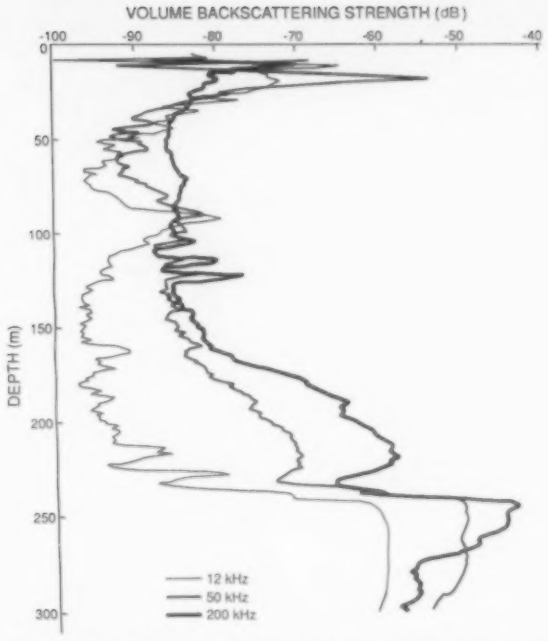


FIG. 3. Profiles of volume backscattering strength (S_v) versus depth at 12, 50, and 200 kHz in central LaHave Basin. Analysed data comprises first 300 s (300 pings) of profile shown in Fig. 1b. Bottom occurs at about 240 m depth.

invariance. Interspersed among the bands are discrete hyperbolic echoes characteristic of fish. These isolated point scatters appear selectively in the wide 12-kHz beam.

Scattering patterns at 50 and 200 kHz are very similar particularly for the deeper diffuse horizons; the green and blue image components superimpose almost exactly to give a fairly uniform shade of greenish blue. For localized scatterer concentrations, the 50- and 200-kHz spatial patterns differ slightly due to the wider 50-kHz transducer beam width. This may be detected as localized variations in the shade of blue or green "fringing" around a more "bluish" core.

In Emerald Basin alone, isolated blue-coloured spatially compact patches appear in the upper half of the water column. The patches typically extend 10 m in the vertical with suggestions of structure above the 3.8-m vertical resolution imposed by the 5-ms, 200-kHz pulse length. Horizontal extents range from 20 m down to 10 m or less, 10 m being the horizontal resolution limit of the 200-kHz beam at approximately 100 m range. The patches are not visible in 12-kHz echograms.

Volume Backscattering Strength (S_v) Profiles (Daytime)

Figures 2 and 3 show S_v versus depth profiles calculated from the first 300 pings of the sections displayed in Fig. 1a and 1b, respectively. Contrasting frequency signatures for the "red" and "blue" deep basin layers are evident from the S_v profiles. The "blue" deep layers scattered strongly at 200 kHz, much less strongly at 50 kHz, and, at 12 kHz, scattered at least 30 dB less intensely than at 200 kHz and are indistinguishable from background. The bottom "red" layer scattered strongly at all three frequencies. In LaHave Basin (Fig. 3), only the topmost portion of the "red" layer escaped being averaged into the intense, variable-depth bottom echo. In the approximate 10-m gap between the base of the blue layer and the top of the bottom red layer, 200-kHz S_v's are depressed up to 12 dB from those immediately above and below. Volume backscattering strength characterization of individual Emerald Basin "blue" compact is addressed in the "interpretation" section.

Shelf Basin Echograms (Nighttime)

Blue layer

About 2 h before local sunset, the LaHave Basin diffuse deep "blue" layer was observed to break into distinct local concentrations forming horizontally semicontinuous horizons with sharply defined upper edges (Fig. 4). The minimum depth to the shallowest blue horizons tended to lessen with time, signifying upward migration. Upward migration of the top boundary was accompanied by a simultaneous general dispersal of the scatterers throughout the water column below. Ascent of the top boundary halted at about 130 m depth. About 15 min before sunset, small isolated swarms appeared to develop out of the uppermost portion of the "blue" horizon and ascended rapidly from about 130 to 80 m over a period of about 8 min. After pausing for about 15 min, these scatterers than rose at 0.04 m/s and distributed themselves diffusely in the top 30 m. After the migrating population had fully stabilized in its nighttime position, it was observed that 200-kHz S_v levels at 180 m, the depth of the daytime "blue" layer maximum, had decreased at least 10 dB from typical daytime values (Fig. 5). Nighttime 200-kHz S_v levels below 100 m were dominated by the diffusely distributed residual of the daytime "blue" layer with a small contribution from isolated scatterers of the vertically redistributed "red" layer (below).

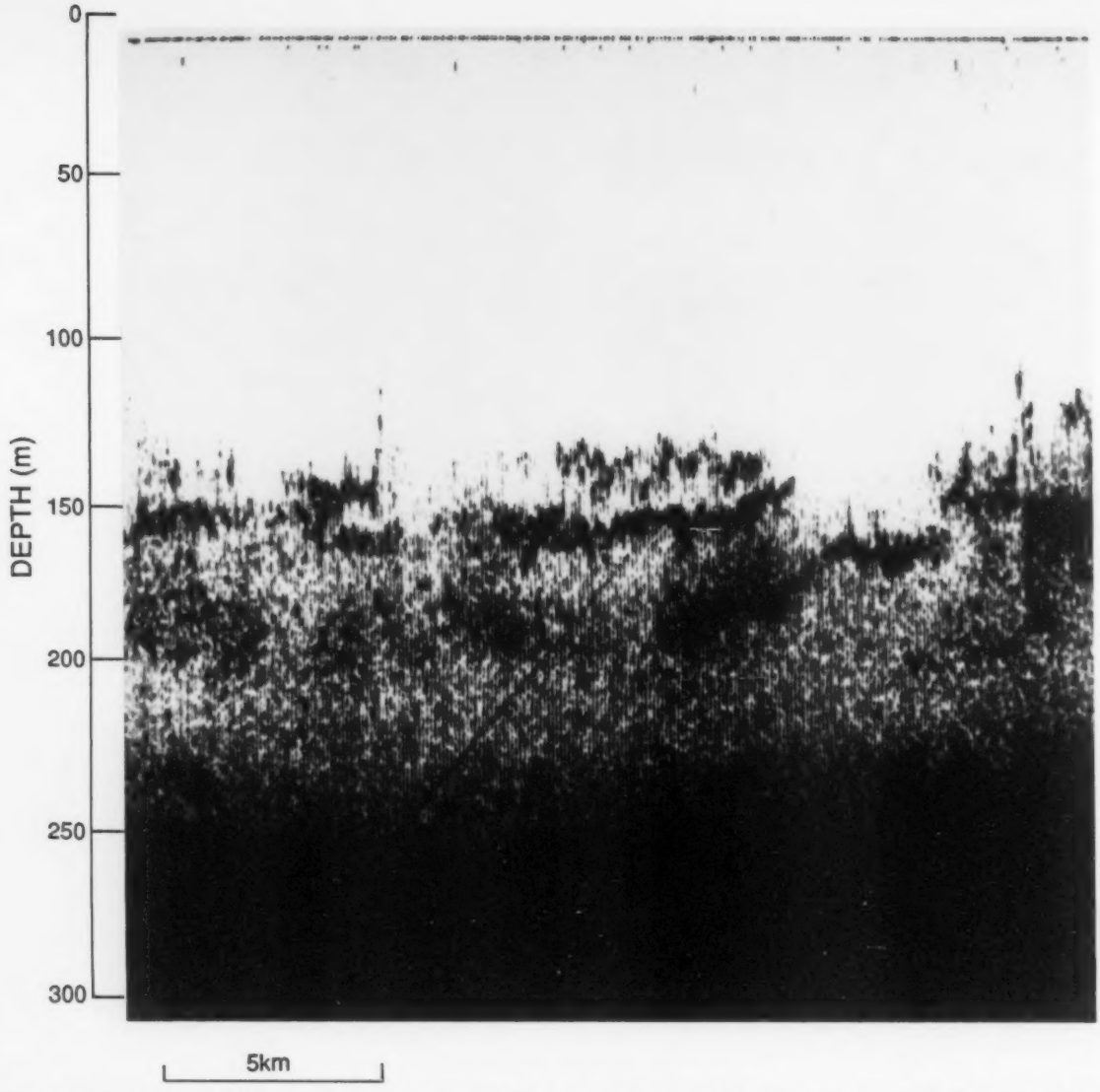


FIG. 4. LaHave Basin towed 200-kHz profile showing appearance of daytime bottom "blue" layer at midwater depths during the ascending segment of its diurnal migration cycle. Section consists of 5120 s of record commencing at 1908 ADT, June 11, 1988, and spanning 43°47.8′N, 63°39.2′W to 43°37.4′N, 63°44.6′W.

Red layer

About 15 min before sunset the LaHave Basin red layer departed the bottom. Its well-defined top boundary moved upward at about 0.03 m/s for 45 min and halted at about 110 m. Below the top boundary, individual red layer scatterers distributed themselves over the entire range from 110 m to bottom. Over the next 50 min a small number of discrete scatterers (parabola-shaped echoes) continued to rise above 110 m to near surface (<15 m depth) at 0.04 m/s (Fig. 6). Observation of the redistributed layer using a higher ping rate and slower ship speed clearly showed that it comprised discrete scatterers. The vertical redistribution occupied about 2 h total. Figure 7 shows a 12-kHz postmidnight S_v profile reflecting the equilibrium nighttime distribution compared with that in daytime. In Emerald Basin, virtually identical distributions and vertical migration patterns to those in LaHave Basin were observed. Strong analogies were apparent in the vertical migration and dispersal of both the "red" and "blue" layers. Differences were that the migration of the "blue" layer was a more irregular phenomenon due to the large-scale "patchiness" observed at midwater depths and that the migration occurred earlier in the day and proceeded more slowly in its initial stages.

Interpretation

General

The "red" and "blue" horizons signify two distinct migrating populations distinguishable by their acoustic spectral responses, echo characters (diffuse versus discrete scatters at midwater depth), diurnal migratory behaviours, and

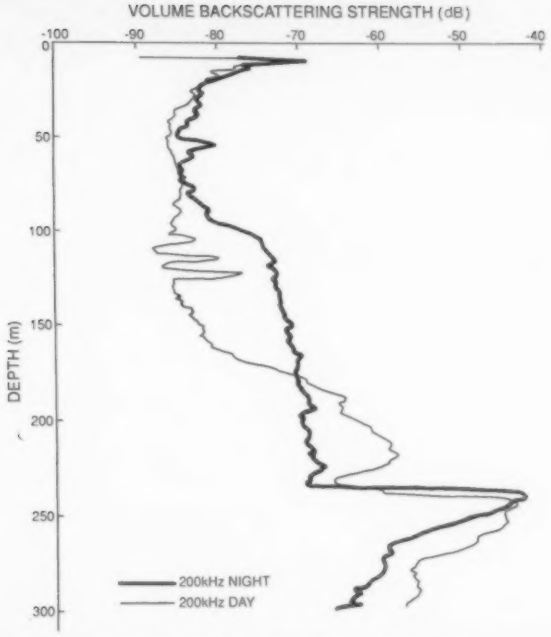


FIG. 5. Comparative 200-kHz day–night volume backscattering strength (S_v) versus depth profiles for central LaHave Basin computed for 300-s (300-ping) averages. S_v averages were initiated at 1531 ADT, June 12 (daytime), and 0023 ADT, June 13 (nighttime). Nominal location 43°43′N, 63°46′W. Bottoms occur near 240 m depth.

aggregation patterns. With reference to BIONESS and trawl sampling, we have attributed the "blue" layer to euphausiids, mainly *M. norvegica*, and the deep daytime "red" layer to fish, principally silver hake.

To enable accurate quantitative interpretation, acoustic volume backscattering strength estimates for Emerald and LaHave basins were further corrected for precise location-specific acoustic absorption losses using BIONESS CTD profiles (Fig. 8). The midwater cold, lower salinity layer was characterized by lower acoustic absorptions than corrected for by the echo sounder in real-time. For 200 kHz, the accumulated S_v corrections were 3.3 dB (two-way transit) at 265 m in central Emerald Basin and 3.7 dB at 240 m in central LaHave Basin. At 50 kHz the corrections were smaller, 0.4 and 0.2 dB, respectively. The 12-kHz effects were negligible.

Red Layers

After correction for anomalous absorption the daytime bottom "red" layers scatter about equally (to within 1 dB) at 50 and 200 kHz and about 7 dB less at 12 kHz. The similarity of 50- and 200-kHz levels points to "geometrical" or "Fresnel" scatterers (Clay and Medwin 1977) with dimensions large compared with the acoustic wavelength.

The postmidnight vertically redistributed red layer was trawled allowing intercomparison with 12-kHz acoustic sampling. Two trawls were examined, the first a trawl at 150 m in central Emerald Basin for which simultaneous acoustics were available and the second a trawl at 135 m in central LaHave Basin with acoustics on another night. Both trawl catches consisted almost exclusively of silver hake with a mean length of 0.27 m. Descriptive trawling parameters and estimates of silver hake abundances, assuming 100% net sampling efficiency, appear in Table 4. Acoustic fish concentrations (Table 4) were derived from relation (1) assuming identical targets and, in the absence of specific target strengths for silver hake, using the general target strength regression given by relation (2) for L = 0.27 m. Similar trawl abundances of about 1 fish/3000 m^3 characterized both basins. The corresponding acoustic abundances of about 1 fish/5000 m^3 were also similar. The fact that the trawl abundances differed from the acoustic abundances by less than a factor of 2 is taken as strong confirmation that the dominant 12-kHz scatterers were silver hake. The differences between the two types of abundance estimate lie within the anticipated uncertainty from use of a non-species-specific target strength regression, ignoring the spread of ensonification angles from the relatively wide beam transducer, and using a mean fish length rather than a length distribution in computing an effective target strength.

The red layers in the 80- to 100-m depth range were also occasionally weakly visible at 50 kHz but rarely at 200 kHz. These layers were normally very thin — their thickness often being pulse length limited at 12 kHz in spite of the broad transducer beam width — suggesting a directive specularlike reflection phenomenon. Echograms showed no suggestion of discreet resolvable organisms. The layers usually displayed good continuity both temporally and spatially and appeared little affected by the normal diurnal biological migration cycles. Since their signatures were also undetected by the BIONESS and BATFISH optical counters and since the layers occurred near density steps at the base of the surface warm layer (Fig. 8), we tentatively attribute them to some physical oceanographic process.

Blue Layers

Comparisons of Emerald Basin S_v profiles from earlier databases with BIONESS catches (Cochrane and Sameoto 1987) showed good correlations between the acoustics and high concentrations (2000/m^3) of the copepods *C. finmarchicus* stage V (2.2 mm length) and *C. hyperboreus* stage IV (3.3 mm length). More extensive analysis now favours an intermixed minority euphausiid population being the dominant scatterer. This conclusion is supported by several convergent lines of evidence. First, earlier acoustic model interpretations required the use of the extreme copepod physical parameters advanced by Holliday and Pieper (1980) while still yielding 200-kHz S_v levels 8 dB less than predicted from measured BIONESS concentrations. Køgeler et al. (1987) subsequently advanced more refined acoustic parameters for *Calanus* which further reduced predicted S_v levels by approximately 16 dB. This made the *Calanus* interpretation untenable. Second, BIONESS observations of the deep *Calanus* layers have shown no diurnal migratory behaviour (Sameoto and Herman 1990) contrary to our acoustic observations of the "blue" layer. Finally, optical zooplankton counter profiles of concentration versus depth for deep *Calanus* have shown subtle but consistent differences from simultaneously recorded acoustic profiles. For instance, Fig. 9 shows simultaneous vertical profiles of 200-kHz volume backscattering strength and optical counts in logarithmic form and gated for the *Calanus* size range. While the optical counter profile below 180 m closely resembles the acoustic S_v profile, the sharp drop at 230 m on the S_v profile is much less pronounced than on the counter profile.

It now appears that the deep blue layer was composed mainly of the euphausiid *M. norvegica*. Approximate acoustic esti-

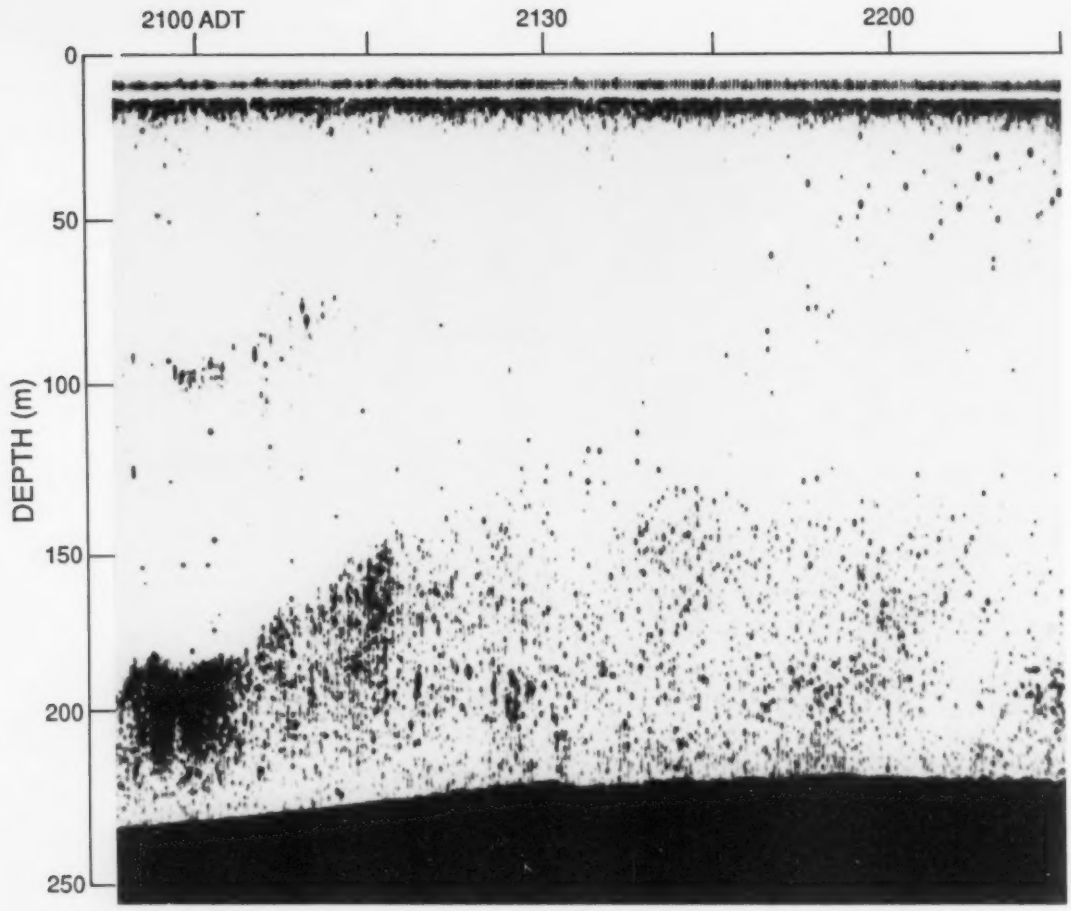


FIG. 6. LaHave Basin towed 50-kHz acoustic section showing upward migration of near-bottom fish layer on approach of darkness. Section consists of 4910 s of record commencing at 2053 ADT, June 11, 1988, and spanning 43°34.4′N, 63°43.7′W to 43°22.3′N, 63°40.5′W. Local sunset under clear skies was observed at 2055 ADT. The corresponding 200-kHz section appears in Fig. 11.

mates of scatter size (next section) lie in the 10- to 30-mm range. June 1988 BIONESS samples contained *M. norvegica* of about 28.0 ± 2.5 mm mean length when considered in total (Fig. 10). Euphausiids were the only abundant sampled component in the BIONESS or IYGPT midwater trawls in roughly this size range. A euphausiid interpretation for the daytime "blue" scattering horizon is also consistent with previously observed euphausiid migratory behaviours. Echograms at 200 kHz show migration from below 150 m to the top 20–30 m (Fig. 11). Previous net sampling in this region has shown *M. norvegica* to vertically migrate into the top 30 m at night (Sameoto et al. 1985; Lewis and Sameoto 1988a, 1988b, 1989). Mauchline and Fisher (1969) reported similar vertical migrations of *M. norvegica* in the Firth of Clyde. The thin multilayered morphology observed of the vertically migrating basin aggregations is consistent with other acoustic observations of euphausiid migration (Sameoto 1976).

Water column echo integration was performed for two LaHave Basin 200-kHz data sets recorded during day and night BIONESS tows, respectively. Each set consisted of three consecutive 300 ping samples gathered on the transects referenced in Fig. 5 and their spatial extensions. Signals from less than 20 m depth were suppressed, as these were dominated by transducer back lobe reflections from the water surface and ship's hull. Anomalous absorption and noise corrections were applied during integration. The column-integrated volume backscattering coefficients averaged to 1.93×10^{-5} in daylight and 6.54×10^{-6} at night. Strict conservation of biomass between day and night would nominally require about 70% of the water column euphausiids to be aggregated in the top 20 m at local midnight. The nighttime S_v profile of Fig. 5 shows no surface concentration in spite of the evening migrations to surface as in Fig. 11. We consider the most probable explanation to be that euphausiids were concentrated near surface but were not detected acoustically due to their systematic vertical orientations in response to escaping ship light at night as demonstrated by Sameoto et al. (1985). Deck lights were on to assist simultaneous BIONESS operations.

Quantitative assessment of the daytime isolated "blue" patches in Emerald Basin (Fig. 1a) presented special problems, since patch spatial dimensions were too small to fill the 50-kHz ensonification volume. This invalidated direct computation of a single patch 50-kHz S_v level using the integrated beam width factor. An alternative statistical technique was employed to define the difference between 50- and 200-kHz effective S_v levels. If a sufficiently large volume of water is acoustically

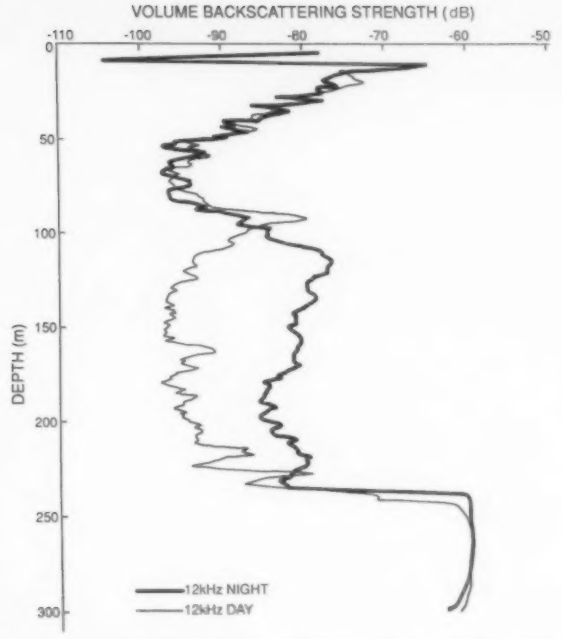


FIG. 7. Comparative 12-kHz day–night volume backscattering strength (S_v) versus depth profiles for central LaHave Basin computed for 300-s (300-ping) averages. S_v averages were initiated at 1531 ADT, June 12 (daytime), and 0023 ADT, June 13 (nighttime). Nominal location 43°43′N, 63°46′W. Bottoms occur near 240 m depth.

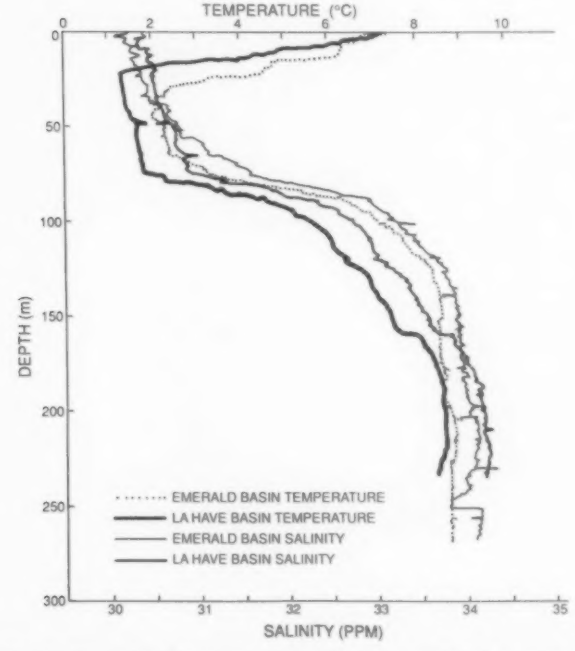


FIG. 8. Representative temperature and salinity profiles from central Emerald and LaHave basins. Measurements were acquired simultaneous with acoustic sections of Fig. 1. using a BIONESS-mounted CTD.

sampled, and if the patches are distributed at random in the horizontal plane within this (otherwise nonscattering) water volume, the difference in mean volume S_v levels converges to the difference in true S_v levels for individual patches. Assessment was carried out by computation of the average vertically integrated 40- to 150-m volume backscattering coefficients (Table 5) over a 1000-ping extended profile consisting of the subsection displayed in Fig. 1a and its continuation. The total transect contained 13 distinct patches. This was followed by inversion to the integrated backscattering coefficients to the difference in effective S_v levels (Table 5). The narrow 5.4°, 200-kHz ensonification cone marginally permitted ping-by-ping S_v measurements for individual patches. The isolated intense patch on the extreme right-hand side of Fig. 1a yielded a peak 200-kHz S_v of -54 dB corrected for anomalous absorption. This value is listed as the "S_v (Peak)" level in Table 5.

Acoustic Modelling of Blue Layer and Patches

Acoustic inputs for inverse modelling of the daytime deep "blue" horizons (Table 5) were derived from three consecutive 300-ping sections for each of Emerald and LaHave basins commencing with those displayed in Fig. 1. Integrated backscattering coefficients were computed over the indicated depth ranges encompassing the "blue" layer to derive comparative 50- and 200-kHz S_v levels.

Camera studies of euphausiids in situ have shown considerable departures from horizontal orientation (i.e. dorsal ensonification). Sameoto (1980) observed correlations between orientational behaviour and time of day. Kristensen and Dalen (1986) proposed a Gaussian distribution of orientations about the horizontal of about 30° standard deviation (SD) in a fixed vertical 2-D plane, a result roughly consistent with Sameoto's (1980) observations at times of predominantly horizontal orientation (1400). We have utilized a zero-mean Gaussian angular distribution of 30° SD as a first approximation for inverse modelling purposes together with euphausiid physical parameters $g = 1.06$ and $h = 1.03$ as suggested by Køgeler et al. (1987). For fluid cylinder modelling, the euphausiid equivalent spherical radius, e.s.r., is empirically related to organism length, L, as explained in the "Methods" section. For *M. norvegica*:

$$(7) \quad L = 17.44\,(\text{e.s.r.})^{1.163} \qquad \text{(MKS units)}.$$

Cylinder length, L, and organism length are assumed identical in our implementation.

Table 6 lists target sizes, concentrations, and biomass per cubic metre computed using the inverse cylinder model and inverse fluid sphere models applied to volume backscattering strength data extracted from Table 5. Note that the 15- and 21-mm euphausiid lengths for the deep basin layer computed from the inverse fluid cylinder model (30° SD), while lying in the euphausiid size range, are less than the 28-mm average length obtained in the BIONESS sampler (Fig. 10). The 53-mm size computed for the patchlike scatterers is considerably larger than organism sizes derived for the deeper layers or observed in BIONESS.

An alternative approach to inverse interpretation of zooplankton acoustics is the use of empirical models based on well-controlled laboratory or field observations of real organisms. Empirical relationships have often been used to functionally relate euphausiid or krill target strength to organism size and occasionally ensonification frequency (P. H. Wiebe, WHOI,

TABLE 4. Midwater trawl and acoustics intercomparison.

	Basin	
	Emerald	LaHave
	Trawl data	
Start time	0326 ADT, June 17, 1988	0016 ADT, June 18, 1988
Trawl depth	150 m	135 m
Trawl length	3.4 km	3.3 km
Sampled Volume	3.4×10^5 m^3	3.3×10^5 m^3
No. of silver hake	111	103
Mean length	0.272 m	0.267 m
Fish concn.	1/3060 m^3	1/3200 m^3
	Comparison acoustic data	
Start time	0326 ADT, June 17, 1988	0023 ADT, June 18, 1988
End time	0406 ADT	0038 ADT
Relevant depth	150 m	135 m
12-kHz S_v level	−78.5 dB	−79.1 dB
Assumed TS	−41.7 dB	−41.7 dB
Acoustic concn.	1/4790 m^3	1/5500 m^3

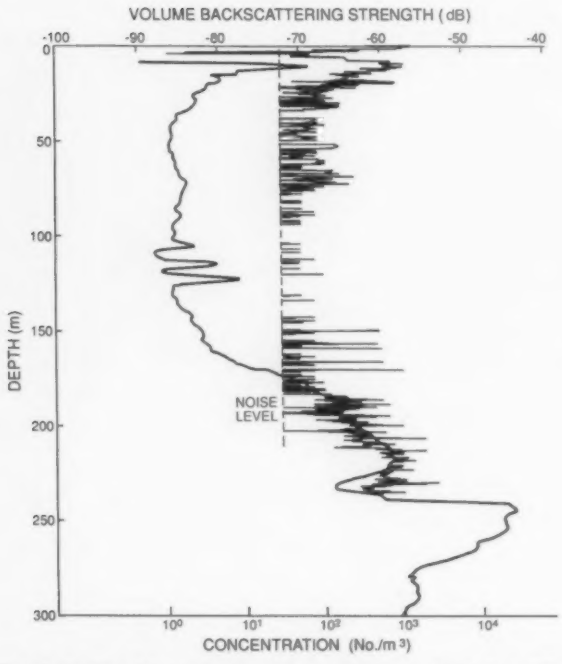


FIG. 9. Profile of log copepod concentration versus depth from BIONESS-mounted optical plankton counter with superimposed simultaneous profile of 200-kHz acoustic volume backscattering strength. Profiles were obtained in central LaHave Basin between 1531 and 1548 ADT, June 12, 1988.

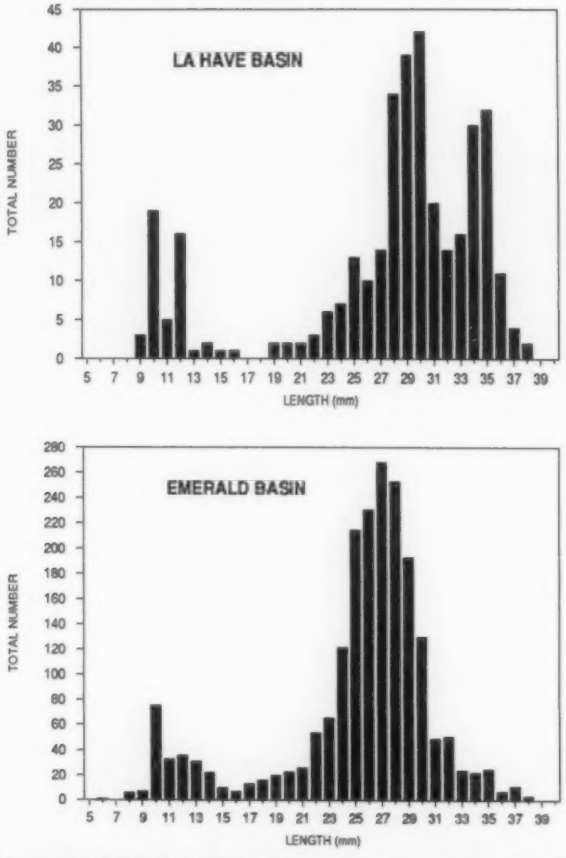


FIG. 10. Body length distribution functions for *M. norvegica* from all June 1988 deep BIONESS tows in LaHave and Emerald basins.

unpubl. data; Greene et al. 1989; Kristensen and Dalen 1986; Richter 1985; and numerous investigations summarized in Thiel 1984 and the POST-FIBEX Acoustic Workshop 1986). Relationships based on observations of real organisms may enable more accurate interpretation of field data than use of reductionist geometry scattering models alone.

We will briefly examine forward application of the above theoretical models in relation to the best controlled experimental measurements. This will furnish additional insight into the relevance of orientational randomness, support the general accuracy of these estimates, and consequently permit the

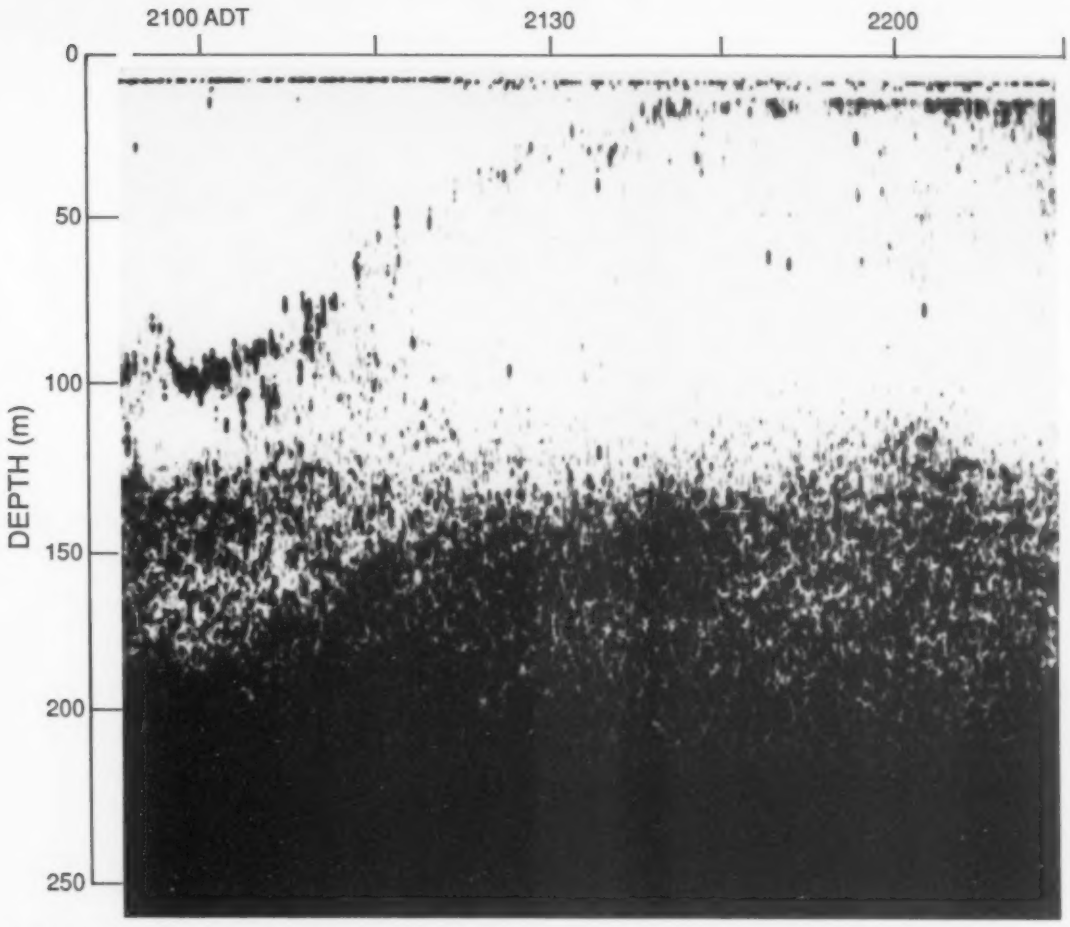


FIG. 11. LaHave Basin acoustic section showing migration of 200-kHz scattering layer from 90–100 m to upper 25 m. Useful acoustic record starts at about 10 m. Section consists of 4910 s of record commencing at 2053 ADT, June 11, 1988, and spanning 43°34.4′N, 63°43.7′W to 43°22.3′N, 63°40.5′W. Image contrasts have been enhanced compared with Fig. 4 to emphasize shallow features. Local sunset under clear skies was observed at 2055 ADT. The corresponding 50-kHz section appears in Fig. 6.

extraction of more reliable quantitative estimates from our database. Table 7 lists theoretical euphausiid target strengths over a range of commonly utilized acoustic frequencies for the Stanton (1989) high-pass fluid cylinder and fluid sphere models. For reference, also included in Table 7 are results from the previously much-utilized Greenlaw (1979) version of the Johnson (1977) high-pass fluid sphere model. Cylinder models treat the case of 0° (broadside) incidence, Gaussian 2-D distributed orientations (30° SD), and completely random orientations in 3-D space. Three lengths of euphausiid have been considered: 15-mm organisms comparable with the smallest from inverse modelling, 28-mm organisms representative of basin BIONESS sampling, and 42-mm organisms representative of adult Antarctic krill (*Euphausia superba*) frequently treated in the literature.

With reference to Table 7 the following conclusions are drawn from forward modelling. First, using the cylinder model, one observes that a rise in target strength with increasing frequency for $ka >> 1$ (in this context, "*ka*" value implies "*a*" being the equivalent spherical radius, not cylinder radius) is characteristic of fixed broadside incidence. However, when a broad ensemble of orientations is considered, as in the Gaussian or completely random models, the target strength for $ka>>1$ becomes essentially independent of frequency analogous to the high-pass fluid sphere models. Second, the dependence of target strength on organism length for the Gaussian and randomly oriented cylinders for $ka>>1$ is approximately 20 log L, again analogous to the fluid sphere and contrasting with the approximate 30 log L characteristic of the broadside ensonified cylinder model. Exact theoretical dependencies of target strength on L at large ka using the Greenlaw and Johnson (1982) *M. norvegica* regression relation (7) are 27.9 log L for the fluid cylinder (broadside incidence) and 17.2 log L for the fluid sphere. Finally, effective target strengths for organisms with orientational SD's of 30° or more are only weakly dependent on the degree of randomness. Note that fluid cylinder effective target strengths decline only about 2 dB on going from a Gaussian 30° SD to complete orientational randomness. The latter observation has two positive implications for acoustic studies of elongate organisms. First, target strengths of real organisms may be relatively insensitive to details of the orientational distribution function as long as a significant degree of randomness is present. Second, finite transducer beam width, which would tend to broaden the orientational distribution function, should

TABLE 5. Emerald and LaHave Basin acoustic parameters for inverse modelling. These values are fully corrected for anomalous acoustic absorption and ambient noise.

IBC_{50}	IBC_{200}	$S_{v\,200-50}^{(Diff.)}$	$S_{v\,200}^{(Peak)}$	$S_{v\,200-50}^{(Diff.\ peak)}$
	Emerald Basin patches, 40–150 m			
1.673×10^{-6}	2.537×10^{-6}	1.81 dB	≈ 54 dB	—
	Emerald Basin, 210–242 m			
1.883×10^{-6}	2.100×10^{-5}	10.47 dB	−58.7 dB	10.95 dB
	LaHave Basin, 190–230 m			
2.800×10^{-6}	1.533×10^{-5}	7.38 dB	−60.9 dB	7.93 dB

IBC_{50}, IBC_{200} Integrated backscattering coefficients at 50 and 200 kHz, respectively

$S_{v\,200-50}^{(Diff.)}$ Effective S_v difference derived from integrated backscattering coefficients: 10 log (IBC_{200}/IBC_{50})

$S_{v\,200}^{(Peak)}$ Peak 200-kHz S_v level, average peak TS from three 300-ping subsections

$S_{v\,200-50}^{(Diff.\ peak)}$ $S_{v\,200}^{(Peak)} - S_{v\,50}^{(Peak)}$

have minimal effect on observed target strengths especially if the transducer half beam width is considerably less than the width of the orientational distribution function. Transducer beam width was not considered in our inverse modelling.

These model observations imply that empirical target strength relationships based on in situ measurements (naturally distributed euphausiid orientations) at one frequency and for one dominant euphausiid length may be used to extrapolate to other frequencies and lengths for $ka >> 1$ by assuming no frequency dependence and an approximate 20 log L length dependence. In fact, this type of extrapolation is quite valid for $ka \geq 2$. Few data from experimental studies of euphausiid scattering can be legitimately compared with these model results or applied to the interpretation of field data due to inadequate experimental controls on organism abundances, unnatural constraints on organism orientations, or insufficient description by which these criteria can be critically evaluated. Living specimens must be free to assume a natural orientation distribution (i.e. untethered and equilibrated to surroundings) and the acoustic data must be selected and processed such that an effective target strength is obtained representative of the full ensemble of natural orientations. On requiring the additional constraints of a reliable number density and size, and observational frequencies lying in the geometric regime where models indicate minimal frequency dependence, the most useful set of measurements to date appear to be the 120-kHz caged *E. superba* target strength measurements by Foote et al. (1990). Consistent with our model studies, we have assumed empirical relations within the geometric regime of the form

$$(8) \quad TS = 20 \log L + B \qquad \text{(MKS units)}.$$

On selecting a subset of Foote et al.'s (1990) published target strength versus size database pertaining to 30- to 32-mm krill, the relevant value of B was calculated to be −48.2 dB. Using the BIONESS sampled 28-mm mean euphausiid length and the above value of B, relation (8) predicted an effective 120-kHz target strength of −79.3 dB. This target strength was also assumed representative of 200 kHz where it compares reasonably well with the theoretical −77.3 dB for a similar sized *M. norvegica* assuming a 30° orientational SD (Table 7). Better agreement was obtained by recomputing the 28-mm cylinder model using Foote et al.'s (1990) measured *E. superba* physical parameters, $g = 1.0357$ and $h = 1.0279$. This yielded a TS = −80.2 dB compared with the empirical TS = −79.3 dB enhancing confidence in both the theoretical and experimental–empirical approaches. It may be of interest that our two-frequency inverse model applied to Foote et al.'s (1990) own discrete *E. superba* TS observations at 38 and 120 kHz selected for 30- to 32-mm euphausiids, and using $g = 1.0357$ and $h = 1.0279$ predicts a length of 29.9 mm and volume density of 1.53/m^3. This is in good agreement with the observed 31.2-mm mean length and 1/m^3 implied volume density.

The most reliable technique for estimating euphausiid concentrations may lie in combining the best aspects of both the theoretical and empirical approaches. Net sampling provides good estimates of size but is plagued by avoidance problems. Acoustics, a remote sensing technique, minimizes short-range avoidance but is less suitable for sizing, since size is a very sensitive function of the two-frequency S_v difference. The close agreement of theoretical and empirical TS's for 28-mm euphausiids above suggests that theoretical TS's are representative of real organisms within 1–2 dB provided reasonable values have been chosen for g and h.

On interpreting peak S_v levels in Emerald and LaHave basins (Table 6) in terms of concentration using relation (1) and the 200-kHz TS value of Table 7 for a 28-mm cylindrical *M. norvegica* of 30° orientational SD, peak concentrations of 72 and 43/m^3, respectively, were obtained. Total water column integrated euphausiid densities (number per square metre), a more meaningful biological parameter, were obtained as the quotient of the vertically integrated volume backscattering coefficient (Clay and Medwin 1977) and the acoustic backscattering cross-section of a typical 28-mm basin euphausiid. This quantity was computed for the three successive 300-ping daytime basin transects beginning with the sections displayed in Fig. 1. Emerald Basin volume backscattering coefficients were integrated between 150 and 245 m including the entire blue horizon to the top of the underlying fish layer but excluding the shallower patchlike features. LaHave Basin coefficients were similarly

TABLE 6. Acoustic inverse model calculations.

Emerald Basin	LaHave Basin
$S_{v\ 200}^{(Peak)} = -58.7$ dB	$S_{v\ 200}^{(Peak)} = -60.9$ dB
$S_{v\ 200-50}^{(Diff.)} = +10.5$ dB	$S_{v\ 200-50}^{(Diff.)} = +7.38$ dB

Incidence angle	Length (mm)	Density (no./m³)	Biomass density (g/m³)
	Emerald Basin		
0°	27.8	8.21	2.26
Gaussian			
5°	21.1	30.2	4.07
10°	18.0	71.3	6.37
20°	15.9	162	10.6
30°	15.3	240	14.0
40°	15.0	298	16.6
Random	14.6	449	23.3
	LaHave Basin		
0°	54.6	0.737	1.56
Gaussian			
5°	29.2	8.96	2.81
10°	24.5	22.6	4.49
20°	21.9	50.9	7.54
30°	21.1	74.2	10.1
40°	20.9	91.2	12.0
Random	20.5	135	16.9

Stanton fluid sphere model		
Length (mm)	Density (no./m³)	Biomass density (g/m³)
	Emerald Basin	
10.2	1070	21.9
	LaHave Basin	
13.0	413	15.9

integrated between 160 and 230 m. Integrated coefficients averaged 2.36×10^{-5} and 1.67×10^{-5} m³ for Emerald and LaHave basins, respectively, after noise and anomalous absorption corrections. These in turn yielded euphausiid column densities of 1250 and 880/m², respectively.

Discussion

The acoustic delineation of basin silver hake and *M. norvegica* scattering layers when combined with BIONESS observations of deep *Calanus* distributions suggests possible diel interactions of the three biological communities. In the daytime, acoustically observed euphausiid layers cooccur with deep copepod concentrations delineated by BIONESS. Nighttime vertical redistribution of the euphausiids and fish result in silver hake, with some sand lance (*Ammodytes americanus*) sampled in near-surface trawls cooccurring with euphausiids. Cooccurrence of prey and predator is a necessary but, of course, not sufficient condition for grazing. Gut content analysis of Scotian Shelf silver hake (Waldron 1988) shows *M. norvegica* to account for 93% of its prey by number. Nighttime equilibrium

TABLE 7. Predicted target strengths (dB) for *M. norvegica* from forward model calculations. Acoustic parameters $g = 1.06$ and $h = 1.03$; ka values, where a refers to the equivalent spherical radius, are given in parentheses for the Stanton fluid sphere model.

Frequency (kHz)	Length (mm)		
	15.0	28.0	42.0
	Stanton fluid cylinder model (high-pass fixed 0° orientation)		
12	−115.1	−101.2	−92.1
50	−90.8	−78.2	−71.1
120	−79.4	−70.4	−65.2
200	−75.7	−67.8	−62.8
420	−72.0	−64.4	−59.5
1200	−67.4	−59.9	−54.9
	Stanton fluid cylinder model (high-pass Gaussian 30° SD)		
12	−116.6	−102.8	−94.1
50	−93.3	−82.4	−76.7
120	−84.4	−77.8	−74.2
200	−82.7	−77.3	−74.0
420	−82.0	−77.0	−73.9
1200	−81.9	−77.0	−73.8
	Stanton fluid cylinder model (high-pass random orientation)		
12	−118.0	−104.3	−95.7
50	−95.2	−84.5	−78.9
120	−86.6	−80.1	−76.6
200	−85.0	−79.6	−76.3
420	−84.4	−79.4	−76.3
1200	−84.3	−79.4	−76.2
	Fluid sphere model (high-pass Stanton formulation)		
12	−115.2 (0.116)	−101.2 (0.199)	−92.3 (0.281)
50	−91.7 (0.484)	−82.5 (0.828)	−78.6 (1.17)
120	−86.3 (1.16)	−81.3 (1.99)	−78.2 (2.82)
200	−86.0 (1.94)	−81.3 (3.31)	−78.2 (4.69)
420	−85.9 (4.06)	−81.2 (6.95)	−78.2 (9.85)
1200	−85.9 (11.6)	−81.2 (19.9)	−78.2 (28.2)
	Fluid sphere model (high-pass Greenlaw formulation)		
12	−115.2	−101.2	−92.1
50	−90.7	−78.7	−73.1
120	−80.9	−75.0	−71.9
200	−79.7	−74.9	−71.8
420	−79.5	−74.8	−71.8
1200	−79.5	−74.8	−71.8

distributions of the silver hake were also consistent with this species' aversion to water colder than about 7°C. It is noteworthy that the sonar-derived vertical upward migration rate for the euphausiid horizon of about 0.04 m/s is consistent with Hardy and Bainbridge's (1954) experimentally measured migration rates for *M. norvegica* of between 0.026 and 0.048 m/s. The acoustically observed euphausiid migration to near surface is also consistent with Sameoto et al.'s (1985) observation of a nighttime echogram light response in Emerald Basin, especially marked in the top 30 m. *Meganyctiphanes norvegica* are known to be attracted to artificial light (Mauchline and Fisher 1969).

Acoustically inferred daytime euphausiid column densities in the neighbourhood of 1000/m² and daytime euphausiid concentrations reaching 10–100/m³ in deeper layers within the Shelf basins greatly exceed BIONESS-inferred concentrations.

For example, BIONESS-derived column densities for *M. norvegica* in Emerald Basin during 1988 only tallied between 10 and 20/m^2 during day and about 40/m^2 at night. Corresponding LaHave Basin column densities were about 5/m^2 in daylight and 40–70/m^2 at night. Nevertheless the acoustic column densities are consistent with the 400–800/m^2 reported by Herman et al. (1991) from 1984–85 BATFISH optical counting in Emerald and LaHave basins. The comparatively low BIONESS column densities imply that euphausiid avoidance of the BIONESS is marked, especially during daylight hours. Daylight net avoidance by the closely related Antarctic species *E. superba* is well known (Everson and Bone 1986). Acoustic observations by Orr (1981) demonstrated that (unidentified) zooplankton can systematically avoid in situ instrumentation at ranges up to 9 m and also move 7–20 m to avoid predators. Avoidance ability may be linked to euphausiid size. For the current June 1988 data set, BIONESS-derived daytime *M. norvegica* concentrations below 200 m were about 0.06/m^3 in Emerald Basin and 0.07/m^3 in LaHave Basin. For an October 1984 data set, Cochrane and Sameoto (1987) reported daytime densities of *M. norvegica* below 200 m in Emerald Basin of about 0.2/m^3. Examination of euphausiid size distributions from these data sets and others (Lewis and Sameoto 1988a, 1988b, 1989) shows a consistent seasonal pattern characterized by a greater proportion of smaller, more juvenile euphausiids in the fall season. The comparatively low June catches may be partly due to more efficient net avoidance by larger adult euphausiids (Sameoto 1980).

Another question is the existence of a reservoir of euphausiids residing directly on the bottom where they remain undetected by our normal sampling techniques. In shallower Scotian Shelf waters, our echograms have revealed euphausiid like scattering layers rising and descending directly from and to the bottom rather than forming a stable layer above bottom during daytime hours. June 1989 video observations in central Emerald Basin with a HYSUB 5000 ROV revealed concentrations of large euphausiids, estimated at between 10 and 100/m^3, within 2 m of bottom, the highest concentration occurring during daylight hours. These observations suggest that a bottom-dwelling community is present in the deep basins but its significance and interaction with the higher water column community is yet to be determined.

An interpretation of the blue patchlike structures in Emerald Basin as schools of small fish is suggested by the size from quantitative modelling. However, euphausiid swarms cannot be ruled out. The number of patches, 13, was low for reliable volume backscattering strength measurements by our statistical technique. Also, any significant unrelated 50-kHz backscattering component arising from the "red" layered complex at similar scattering depths would have tended to bias inverse modelling toward larger sizes. Note that BIONESS euphausiid samples from Emerald Basin (most samples come from night tows) showed a distinct component with lengths around 35 mm which is not present in LaHave Basin. The daylight patches which appeared only, or at least much more abundantly, in Emerald Basin may be swarms of these larger euphausiids. However, the present database is only sufficient to pose, not resolve, the question.

In regard to quantitative acoustic assessment itself, we draw attention to several observations. First, while accurate prediction of silver hake abundances from acoustics requires an equally accurate estimate of effective target strength, the ratio of trawl to acoustically derived fish abundances for both basins agreed to within 10%. Therefore, even a relatively unsophisticated echo sounding system, using a broad single-beam transducer, hull-mounted, might be useful for spatially extrapolating silver hake trawl surveys. Second, our acoustic modelling of "effective" euphausiid target strengths indicated an approximate 20 log L dependence of target strength on organism length in the geometric regime using the fluid cylinder model and a natural distribution of target orientations. This dependence is analogous to that of the traditional fluid sphere model.

Recent experimental target strength measurements for a range of zooplankton sizes and species have indicated a close to 30 log L target strength dependence on length. Richter's (1985) dorsal aspect experimental measurements yielded about 26.7 log L when combined with the Greenlaw and Johnson relation (7), while the Greene et al. (1989) and P. H. Wiebe (WHOI, unpubl. data) dual-beam zooplankton measurements yielded 31.1 log L. Two factors contributing to this apparent discrepancy should be considered. First, the very high acoustic frequencies utilized, 1200 and 420 kHz, respectively, resulted in most observations being made at high ka values where target granularity might reasonably be resolved. Resolved granularity could yield a backscattering cross-section proportional to volume or a 30 log L target strength dependence. Second, euphausiid sized targets were reportedly tethered in approximate dorsal aspect in both referenced studies. The cylinder model predicts a 30 log L target strength dependence for fixed dorsal aspect. We feel that these measurements, while favouring a 30 log L dependence at high ka values, do not contradict a close to 20 log L dependence at $ka \leq 4$ for euphausiid ensembles containing a natural distribution of organism orientations.

Our acoustic modelling of euphausiid scattering has assumed a Gaussian distribution of orientations about the horizontal. Photographic studies, especially those of Sameoto (1980), have shown that euphausiid orientations, at times, scatter about a nonhorizontal tilt angle. We have not modelled such cases but suspect that the effect on target strength is minor provided the above tilt is somewhat less than the width of the tilt angle distribution. Sameoto's observations of large systematic tilt angles were confined to shallow layers at night. At the time of these observations the effect of ship's lights on euphausiid orientation was not appreciated. Consequently we believe that the clustering of naturally occurring tilts scattering around a significant nonhorizontal orientation is not firmly established. This question should be pursued both theoretically and experimentally.

An important question for future acoustic studies in the Shelf basins is quantitative acoustic assessment of the deep copepod community. The random cylinder scattering models show S_v levels from the euphausiid component to reach their "high-pass" plateau level at about 100 kHz. Predicted S_v levels for deep basin copepods at 1200 kHz, assuming 3.3-mm body length (an extreme upper bound assuming entirely *C. hyperboreus* stage IV), $g = 1.00$, $h = 1.03$ (Køgeler et al. 1987), and extreme maximum BIONESS observed concentrations of 2000/m^3 (Sameoto and Herman 1990), are -72 and -73 dB using the randomly oriented high-pass cylinder and sphere models, respectively (considering *C. hyperboreus* IV is usually a minority fraction, the effective scatterer concentration is more likely several times less than 2000/m^3). The copepod S_v levels should not significantly increase with increasing acoustic frequency. In the presence of euphausiid-origin S_v levels often exceeding -60 dB, special signal processing will be required to recover the copepod background. Direct resolution and gating out of discreet euphausiid echoes at close range will be

attempted using a deep-towed sonar system now finishing development at the Bedford Institute of Oceanography.

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Contrasting Trophic Level Interactions in Lake St. George and Haynes Lake (Ontario, Canada)

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Lafontaine, N., and D. J. McQueen. 1991. Contrasting trophic level interactions in Lake St. George and Haynes Lake (Ontario, Canada). Can. J. Fish. Aquat. Sci. 48: 356–363.

Two small, adjacent kettle lakes in southern Ontario were sampled during spring and summer 1987. The data comprised weekly samples of zooplankton and water chemistry, monthly diel assessments of the densities of pelagic fish and zooplankton found at 1-m depth intervals in the water column, and an annual mark and recapture assessment of the entire fish population. The two lakes had very different community structures. Haynes Lake was characterized by high piscivore numbers, few planktivores, a relatively large assemblage of large bodied zooplankton, low chlorophyll *a* concentrations, and clear water. Lake St. George had a lower piscivore to planktivore ratio, smaller zooplankton, more chlorophyll *a*, and murkier water. Comparisons of trophic level biomasses for the two lakes suggested that in both communities, the relationships between piscivores and planktivores and between planktivores and zooplankton were strongly correlated with predator abundances. In the more oligotrophic community (Haynes Lake) this influence extended weakly to the phytoplankton, but in the more eutrophic system, little of the variability in chlorophyll *a* with respect to total phosphorus could be explained by total zooplankton (or *Daphnia*) abundance. This suggests that for freshwater pelagic communities, top-down effects may be stronger in more oligotrophic systems.

Deux petits lacs de kettle ovoisinants du sud de l'Ontario ont été échantillonnés au printemps et à l'été de 1987. Les données recueillies comprennent des échantillons hebdomadaires du zooplancton et des caractéristiques de l'eau, des évaluations nycthémérales mensuelles de la densité des poissons pélagiques et du zooplancton présents à des intervalles de profondeur de 1 m, ainsi qu'une évaluation annuelle par marquage et recapture de toute la population de poissons. La structure des communautés des deux lacs s'est révélée très différentes. Ainsi, le lac Haynes abritait un nombre élevé de poissons piscivores, peu de poissons planctivores, un assemblage relativement important de gros zooplancton, des concentrations faibles de chlorophylle *a* et des eaux claires. Par contre, le lac St-George avait un plus faible rapport entre les piscivores et les planctivores, du zooplancton de plus petite taille, une concentration plus élevée de chlorophylle *a* et des eaux plus troubles. Des comparaisons des biomasses selon le niveau tropique dans les deux lacs portent à croire que les relations entre, d'une part, les piscivores et les planctivores et, d'autre part, les planctivores et le zooplancton était en nette corrélation avec l'abondance des prédateurs. Dans le lac Haynes plus oligotrophe, cette influence touchait peut le phytoplancton, mais dans le lac St-George plus eutrophe, peu de la variabilité de la teneur en chlorophylle *a* par rapport à la teneur totale en phosphore pouvait être expliquée par l'abondance totale du zooplancton (ou *Daphnia*). Ceci porte à croire que les incidences descendantes peuvent être plus marquées dans les systèmes oligotrophes dans le cas des communautés dulçaquicoles pélagiques.

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For several years, limnologists have explicitly recognized that the biomass of trophic levels in the pelagic zones of lakes is determined by the combined and interacting effects of resource availability and predator abundance (Carpenter et al. 1985; McQueen et al. 1986). Empirical models that relate the abundances of phytoplankton, zooplankton, planktivores, and piscivores to their respective resource bases have been reviewed in McQueen et al. (1986) who found that these "bottom-up" correlations between the mean annual biomasses of adjacent trophic levels were statistically significant at the bottom of the food chain, but were weak at the top. McQueen et al. (1986) also developed a second series of trophic level correlations based on enclosure manipulations of predator abundances and found that these top-down relationships tended to be significant at the top of the food chain and weaken towards the bottom. McQueen et al. (1990) suggested that (1) maximum trophic level biomasses are determined by resource availability, (2) within these resource-constrained limits, realized biomasses may be determined by predators, and (3) predator impacts are strongest at the top of the food chain and weaker towards the bottom.

These predictions have been tested by many lake, pond, and enclosure manipulations (reviewed by McQueen et al. 1989; McQueen 1990) and there is now evidence to support the prediction that the impacts of changes in the piscivore community are damped as they pass from the top to the bottom of the food web. There is also a great deal of disagreement about the degree of damping and about the mechanisms that are involved. For example, Carpenter (1989) has suggested that the magnitude of piscivore manipulation is important and that order of magnitude changes in piscivore biomass are required for predictable cascading impacts on phytoplankton. Also, Timms and Moss

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TABLE 1. Morphometric characteristics of Lake St. George and Haynes Lake.

	East basin of Lake St. George	Haynes Lake
Total area (m^2)	58 547	23 327
Maximum depth (m)	16	16
Mean depth (m)	6.1	6.9
Volume (m^3)		
0–5 m	253 758	94 056
5–8 m	35 702	36 841
8–16 m	64 352	31 759

(1984), Balls et al. (1989), Irvine et al. (1989), and Meijer et al. (1989) (reviewed in McQueen 1990) have shown that lake depth is important and that complete fish removal in shallow lakes is associated with significant decreases in phytoplankton biomasses and improvements in water clarity. Finally, McQueen et al. (1986) and Vanni et al. (1990) have suggested that there may be an interaction between nutrient availability and predator impact so that the top-down cascade is strongest in more oligotrophic systems and more damped in more eutrophic systems.

The objective of our study was to assess some of these predictions by investigating the relative influences of predators and resource availability on the pelagic community structure of two small kettle lakes in southern Ontario. The two lakes that we chose for our study superficially appeared to be very similar. They are separated by less than 0.5 km, share the same geological structure, and are surrounded by farm land. There were also differences. Haynes Lake has more steeply sloping sides and is more oligotrophic. Haynes Lake has also long been known to be much clearer (Wile and McCombie 1972) than Lake St. George and to have a daphnid population comprising much larger individuals (Stirling and McQueen 1987). These differences implied that the Haynes Lake community should feature stronger cascading impacts from fish to phytoplankton (Shapiro et al. 1975; Shapiro and Wright 1984; Carpenter et al. 1985, 1987; Carpenter 1989) and that differences in community structure might be influenced by basin morphology and trophic status (nutrient availability). We tested these predictions by assessing the biomasses at each trophic level and by comparing the results between lakes and with independent estimates of trophic level biomass derived from the literature.

Methods

Both lakes are situated on the Oak Ridges Moraine 30 km north of Toronto. Both are kettle lakes, 16 m deep, are dimictic, and have clinograde oxygen profiles. Haynes Lake is smaller than Lake St. George (Table 1) and has steeply sloping sides and less surface area over water <4 m deep.

Samples for zooplankton, phytoplankton, and water chemistry were taken each week from April through September. Detailed descriptions of sampling methods are given in McQueen et al. (1986, 1989) and Johannes et al. (1989). In brief, zooplankton samples were taken at 1, 3, 5, 8, 12, and 14 m using a 35-L Plexiglas plankton trap and combined samples were used to estimate abundances of cyclopoid copepods, calanoid copepods, *Daphnia*, bosminids, *Ceriodaphnia*, nauplii, rotifers, and *Diaphanosoma*. For the Lake St. George samples, a computer-assisted caliper device (Sprules et al. 1981) was used to estimate the biomasses in each group. For Haynes Lake, the caliper device was used every fourth week to estimate mean individual weights for each taxonomic group, and for the other three weeks, biomasses were estimated by multiplying weekly population counts by the monthly mean individual weights for each taxonomic group. In addition to weekly samples noted above, once each month, diel zooplankton samples were taken at 1-m depth intervals every 6 h over 24 h. For these samples the caliper device was always used to estimate biomasses and the samples were combined to produce separate diel estimates for the epilimnion (1–4 m), metalimnion (5–6 m), and hypolimnion (7–14 m). *Chaoborus* were counted and measured separately at each 1-m interval.

Chlorophyll *a* and water chemistry data were collected from the epilimnion using an integrated sampler. Analyses were carried out by the technical staff at the National Water Research Institute (Canada Centre for Inland Waters) using the methods of Aspila et al. (1976).

Fish Assessments

The fish community was assessed with (1) a spring mark–recapture program (Young 1989; Johannes et al. 1989) and (2) monthly series of diel purse seine samples.

The objective of the mark–recapture program was to estimate the spring abundances of piscivores and planktivores prior to the production of young. At Haynes Lake, the assessment was conducted from April 8 to May 1, 1987. Fish were collected using three trapnets (6-ft box), a purse seine (30 × 10 m), and a beach seine (30 × 2 m). Fish were marked with either an upper or lower caudal clip or they were tagged with numbered plastic suture tags measuring (10 mm long × 2 mm in diameter). Numerical estimates of population size were made using sequential mark–recapture methods (Ricker 1975). During the sampling period there were 17 sequential mark–recapture cycles, each comprising at least three trapnet sets and 10 beach seine sets. Each cycle involved 10–15 person-hours of effort. At Lake St. George, the assessment was made from April 7 to April 18, 1987, and comprised six mark–recapture cycles, each requiring 24 h and 30–40 person-hours of effort. The gear comprised four trapnets (6-ft box), one purse seine (30 × 10 m), one beach seine (30 × 2 m), and occasional use of an electrofishing boat. The fish were tagged as described above and estimates were made using sequential mark–recapture techniques (Ricker 1975).

The objective of the diel fish assessment program was to assess fish abundances in the pelagic zone once each month at (1) midday, (2) late afternoon, (3) dusk, (4) night, and (5) dawn. During each sampling period, fish were sampled at three deepwater stations using a dark green 30 × 10 m purse seine with a 1-mm mesh size. All of the fish caught were identified to species, and lengths (total length in millimetres) and weights (fresh weight in milligrams) were recorded. From each catch, approximately 20 of each species (when available) were kept for stomach analysis and the rest were released.

Results

Fish Estimates

Both the planktivore and piscivore communities were more diverse and much more abundant in Lake St. George than in Haynes Lake (Table 2). The piscivore community at Lake St. George comprised *Esox lucius*, *Micropterus salmoides*, and *Stizostedion vitreum*. The planktivore community was

TABLE 2. Fish abundance estimates (numbers/ha) for Lake St. George and Haynes Lake. Estimates are based on mark–recapture studies conducted during the spring of 1987. The numbers in parentheses are 95% confidence intervals. Asterisks denote facultative benthivores.

	Lake St. George	Haynes Lake
Planktivores		
Golden shiner	2138 (1848–2507)	To few to estimate
Yellow perch	3557 (3028–4309)	80 (27–400)
Rock bass*	171 (58–858)	Absent
Bluntnose minnow	233 (no estimate)	Absent
White sucker*	Absent	18 (11–30)
Pumpkinseed*	1321 (940–1855)	2515 (no estimate)
Piscivores		
Largemouth bass	662 (454–963)	27 (7–269)
Northern pike	45 (26–85)	29 (13–79)
Walleye	45 (no estimate)	Absent
Brown bullhead*	41 (20–104)	662 (360–1410)
Planktivore total	7420	2613
Piscivore total	766	718
Piscivore to planktivore ratio	0.10	0.27
Ratio excluding rock bass, white sucker, pumpkinseed, and brown bullhead	0.13	0.70

dominated by *Perca flavescens* and *Notemigonous chrysoleucas*. In Haynes Lake, the piscivores included *E. lucius* and *M. salmoides* and the only planktivore that was encountered in numbers large enough to yield a mark–recapture estimate was *P. flavescens*. Both lakes had sizeable populations of *Lepomis gibbosus* and *Ictalurus nebulosus*, but these species were seldom captured in the pelagic zone (defined here as the zone outside the 5-m contour) of either lake. The numerical piscivore to planktivore ratio was 0.10 for Lake St. George, 0.27 for Haynes Lake (Table 2), suggesting that piscivores were relatively more abundant in Haynes Lake.

The diel purse seine net samples were taken at several time periods once during each month (Tables 3 and 4). For both lakes, the catches were highest during July, but the species compositions encountered in the two lakes were completely different. In Haynes Lake, almost all of the fish that were captured were young-of-the-year (YOY) *M. salmoides*. In Lake St. George, the dominant species were *N. chrysoleucas* and *P. flavescens*. At Lake St. George, *M. salmoides* were never observed in the purse seine catches. At Lake St. George the mean catch per purse seine set was 24.5 fish and at Haynes Lake was 39.4 fish, but almost all of the Haynes Lake fish were captured in one net set at 09:00 on July 10 and all were YOY *M. salmoides*. This is reflected in the mean biomass per set which was much higher in Lake St. George (198.6 g per set, Table 3) than in Haynes Lake (70.3 g per set, Table 4).

Zooplankton

Biomass estimates based on the means of the diel samples collected during June through September (Fig. 1) suggested that biomass was consistently higher in Lake St. George (Lake St. George = 4591 μg/L and Haynes Lake = 3148 μg/L) but that mean individual body size was larger in Haynes Lake (Lake St. George = 0.39 mm and Haynes Lake = 0.49 mm). This was especially obvious for *Daphnia* where the mean body length was 0.85 mm for Lake St. George and 1.19 mm for Haynes Lake (Wilcoxin test, $p < 0.001$). The abundance of the major taxonomic groups was similar for both lakes (Fig. 2 based on weekly counts), but because individuals were larger in Haynes Lake, the biomass contributions were different. In Lake St. George the contributors to biomass were daphnids (48%), cyclopoids (29%), calanoids (12%), and bosminids (4%). In Haynes Lake, the most important contributors to biomass were daphnids (81%), calanoids (12%), and cyclopoids (4%). The two lakes were also very different with respect to *Chaoborus* (Fig. 3). In Haynes Lake, *C. punctipennis* appeared in the plankton earlier and was much more abundant than in Lake St. George. This species also contributed more to total biomass in Haynes Lake (Fig. 1).

Bottom-up Relationships

In Haynes Lake, total phosphorus and chlorophyll *a* concentrations were lower than in Lake St. George and Secchi depth was greater (Tables 5 and 6). The two lakes were clearly separated (ANCOVA, $p < 0.05$) on a chlorophyll *a* versus total phosphorus regression plot (Fig. 4, bottom panel), with Haynes Lake having less total phosphorus and much less chlorophyll *a*. Also, for Haynes Lake, the mean summer ratio of chlorophyll *a* to total phosphorus (both measured as micrograms per litre) was 0.10 whereas at Lake St. George it was twice as great (0.21) and the weekly transformed (arcsine square root) ratios were significantly different (Mann Whitney test, $p < 0.001$) for the two lakes. Additionally, the two lakes separated on a zooplankton versus chlorophyll *a* plot, with Haynes Lake having less zooplankton biomass per unit chlorophyll *a*. For Lake St. George, the mean annual zooplankton biomass to chlorophyll *a* ratio was 635 and for Haynes lake it was 4340. The transformed ratio estimates (based on weekly samples) were also significantly different (Mann Whitney test, $p < 0.009$).

Zooplankton and Phytoplankton

For both Lakes there were negative relationships between the ratios of chlorophyll *a* to total phosphorus and *Daphnia* biomass (Fig. 5), but neither was significant ($\alpha = 0.05$). Also, the relationships between the ratios of chlorophyll *a* to total phosphorus and total zooplankton biomasses were not significant, and the relationships between the ratios and zooplankton biomasses lagged by a week were not significiant.

Discussion

Fish Community Structure

Lake St. George had greater abundances of both piscivores and planktivores (Tables 2, 3, and 4), but Haynes Lake had a higher piscivore to planktivore ratio. Our data suggest that these differences in fish community structure are determined in part by several interacting factors including predation, nutrient availability, and lake morphometry.

For many years, fisheries biologists (Loftus and Regier 1972; Colby et al. 1972; Leach et al. 1977 and Persson 1983) have suggested that there is a successional pattern of fish community structure along trophic gradients. The "holistic" model for freshwater community structure (Persson et al. 1987) invokes this pattern to predict that with increased nutrient availability, we can expect a unimodal rapid increase and then a gradual decrease in piscivore numbers and that these changes at the top of the community "cascade" (Carpenter et al. 1985) through

TABLE 3. Lake St. George fish catches over 24 h during four assessment periods. Number of sets refers to the number of sets using a 30 m long × 10 m deep purse seine. Total catch is the catch per time period. Species caught is the number of individuals of each species caught per day (GS = golden shiner, YP = yellow perch, PS = pumpkinseed) and numbers in parentheses are mean weights. Biomass per set is the average weight of fish caught (excluding larval fish) per set.

Date	Time	Number of net sets	Total catch	Mean catch/set	Species caught (mean wt, g)	Biomass per set (g)
June 13	16:00	3	0		17 GS (10.7)	
	21:00	2	41		1 YP (4.5)	
	03:00	3	14	6.8	36 larval fish	23.3
July 8–9	07:00	3	116		163 GS (17.5)	
	14:30	1	197		194 YP (5.8)	
	21:00	1	155		188 larval fish	
	01:00	2	78	78.0		568.2
August 10–11	14:30	3	73		49 GS (15.6)	
	19:00	3	0		79 YP (10.0)	
	23:30	3	22			
	04:00	3	28			
	08:00	3	5	8.5		103.6
September 9–10	15:30	3	0		53 GS (21.4)	
	18:30	3	0		2 YP (6.9)	
	22:30	1	18		1 PS (41.0)	
	03:00	2	33			
	10:00	3	5	4.7		99.1

Mean catch per set = 24.5 fish
Mean biomass per set = 198.6 g

TABLE 4. Haynes Lake fish catches over 24 h during four assessment periods. Number of sets refers to the number of sets using a 30 m long × 10 m deep purse seine. Total catch is the catch per time period. Species caught is the number of individuals of each species caught per day (GS = golden shiner, YP = yellow perch, PS = pumpkinseed) and numbers in parentheses are mean weights. Biomass per set is the average weight of fish caught (excluding larval fish) per set.

Date	Time	Number of net sets	Total catch	Mean catch/set	Species caught (mean wt, g)	Biomass per set (g)
June 13	03:00	3	6		239 larval fish	
	07:00	3	247	42.1	14 YP (4.5)	10.5
July 10–11	09:00	3	1186		1257 LB (1.7)	
	21:00	3	45			
	23:00	3	10			
	02:00	2	16	114.2		194.2
August 12–13	17:00	3	0		8 LB (4.9)	
	19:00	2	1		1 PS (114.0)	
	22:30	3	5			
	02:30	3	3	0.8		13.9
September 7–8	16:30	1	2		4 LB (7.1)	
	19:00	3	0		4 PS (150.0	
	22:00	1	0			
	02:00	3	2			
	09:00	2	2	0.6		62.8

Mean catch per set = 39.4 fish
Mean biomass per set = 70.3 g

the food web to effect all other trophic levels. The holistic hypothesis predicts that as nutrient availability increases along a trophic gradient, planktivores decrease and then gradually increase, zooplankton increase and then decrease relative to nutrient supply, and phytoplankton are held at low levels when zooplankton are abundant and increase as zooplankton abundance decreases relative to nutrient supply. These patterns may account for some of the differences in fish community structure observed at Lake St. George (where nutrient concentration is higher and the piscivore to planktivore ratio

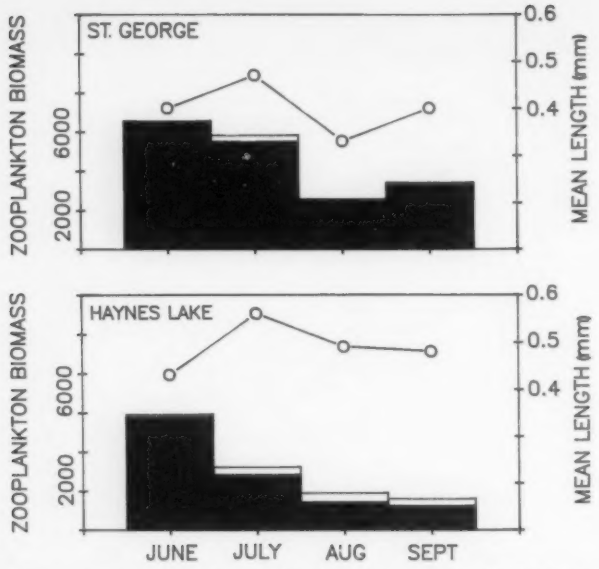

FIG. 1. Biomasses (μg fresh wt/L) of total zooplankton (shaded portion of each bar) and *Chaoborus* (open portion of each bar) in Lake St. George and Haynes Lake. The line diagram in the upper quadrant of each panel represents the mean length of all zooplankton (excluding *Chaoborus*). The data are based on five diel samples taken once each month.

low) and at Haynes Lake (where nutrient concentrations are low and the piscivore to planktivore ratio high), but factors other than lake trophy are also important.

Haynes Lake has steeply sloping sides which offer little cover for YOY fish. When we were marking and recapturing the fish populations in Haynes Lake, we noted the presence of a number of potential planktivores including *P. flavescens*, *N. chrysoleucas*, and *Fundulus diaphanus*. Later, during summer near the shore, we observed a few small schools comprising juveniles from these species, but they soon disappeared and were never found in our pelagic purse seine samples. Johannes et al. (1989) noted that at Lake St. George, YOY *M. salmoides* may have contributed heavily to juvenile planktivore mortality. At Haynes Lake, the total bass population was smaller than at Lake St. George (Table 2), but during June and early July, YOY *M. salmoides* were very abundant (Tables 3 and 4). It therefore seems likely that lack of suitable refuges for planktivores, combined with high numbers of YOY *M. salmoides*, may have contributed significantly to the maintenance of the "piscivore-heavy" fish community that is observed at Haynes Lake.

Zooplankton

At Lake St. George, the top-down effects of low piscivore to planktivore ratios and high planktivore abundances as well as the bottom-up effects of high total phosphorus and chlorophyll *a* concentrations combined to produce large biomasses of small zooplankton. At Haynes Lake, the patterns were reversed (a high piscivore to planktivore ratio and low total phosphorus and chlorophyll *a* concentrations) and the zooplankton community comprised larger individuals and less total biomass. Also at Haynes Lake, the ratios of zooplankton biomass per unit chlorophyll *a* were higher than the ratios found at Lake St.

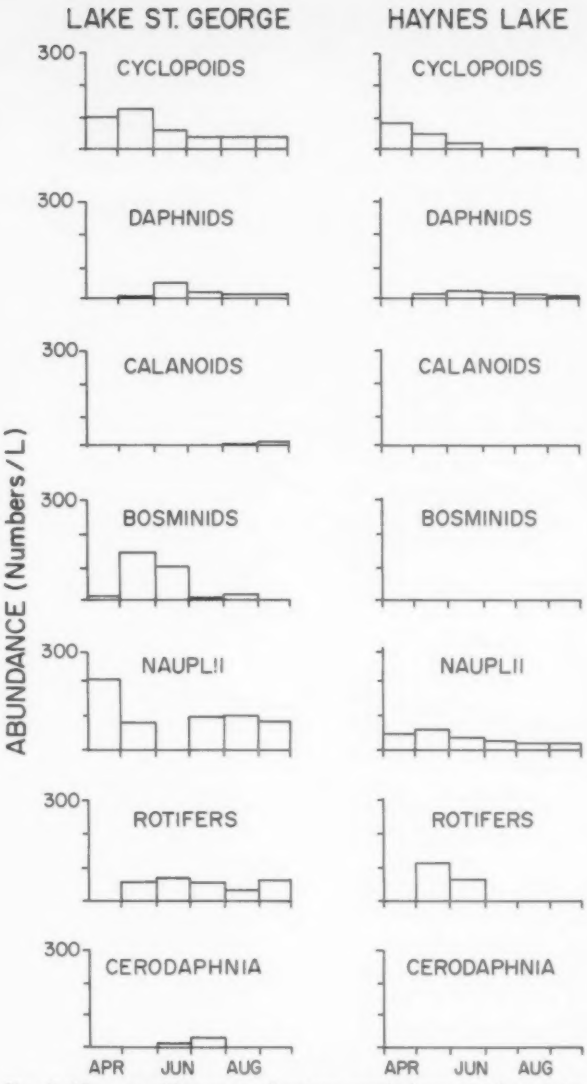

FIG. 2. Mean monthly counts of various zooplankton taxa from Lake St. George and Haynes Lake. The bars represent the means of weekly samples taken throughout the summer.

George, and the Haynes Lake zooplankton biomasses per unit chlorophyll *a* were much higher than the mean annual biomasses reported in the literature (Fig. 4, top panel) (McQueen et al. 1986).

All of these observations are in strong agreement with the top-down predictions of a number of models (reviewed in Northcote 1988) including the holistic model (Persson et al. 1987), the size efficiency model (Brooks and Dodson 1965), the biomanipulation model (Shapiro et al. 1975), and the cascading trophic interaction model (Carpenter et al. 1985). They are also in agreement with the bottom-up predictions of a number of studies (McCauley and Kalff 1981; Mills and Schiavone 1982; Canfield and Watkins 1984; Hanson and Peters 1984) that have demonstrated strong relationships between zooplankton biomass and measures of resource availability (total phosphorous or chlorophyll *a*). Together, these observations support the generalizations formulated by McQueen et al. (1986) and

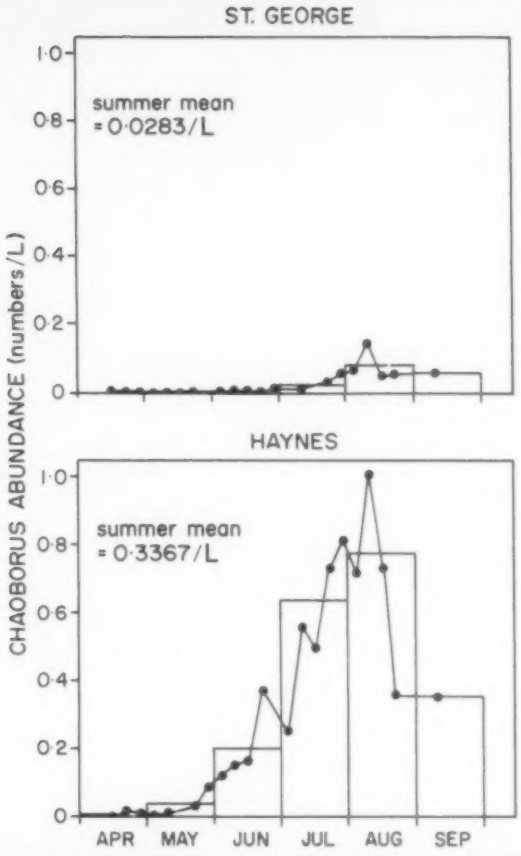

FIG. 3. Mean monthly counts of *C. punctipennis* from Lake St. George and Haynes Lake. The bars represent the monthly means of weekly samples taken throughout the summer. The lines are plotted through the weekly numerical estimates.

suggest that total zooplankton biomasses are most strongly influenced by resource availability, but that species composition and size structure are most strongly influenced by predator abundance.

Phytoplankton

For Haynes Lake, the weekly measures of chlorophyll *a* (Fig. 4b) were lower than the concentrations found at Lake St. George and much lower than the values predicted from chlorophyll *a* versus total phosphorus regressions reviewed by McQueen et al. (1986) (Fig. 4, bottom panel). Also the Haynes Lake ratios of chlorophyll *a* to total phosphorus were significantly lower than the ratios for Lake St. George. These results suggest that at Haynes Lake, the top-down effects of grazing by smaller biomasses of larger zooplankton are stronger than the effects of grazing at Lake St. George where total zooplankton biomasses were higher, but individual body weights smaller.

Conclusions

Comparisons of the pelagic community structure patterns at Lake St. George and Haynes Lake suggest that lake basin morphometry and availability of suitable refuges for YOY planktivores play an important role in structuring fish communities. Also that fish community structure has a strong impact on zooplankton species composition and size structure, but a weaker effect on total zooplankton biomass which is strongly influenced by resource availability. Finally, that zooplankton communities comprising large grazers have more impact on phy-

TABLE 5. Water chemistry data for Lake St. George. Total phosphorus and chlorophyll *a* were collected in the epilimnion (0–4 m).

Date	Total phosphorus (μg/L)	Chlorophyll *a* (μg/L)	Secchi depth (m)
April 15	48	11.3	1.55
25	41	12.1	1.15
29	34	9.7	1.02
May 5	37	7.2	1.15
13	33	11.5	1.05
20	23	5.2	1.15
26	28	1.9	1.95
June 2	24	0.1	2.90
9	23	4.1	1.50
16	25	2.8	1.10
22	17	5.7	1.60
30	33	2.7	1.95
July 7	19	6.7	2.00
21	26	7.5	1.15
28	—	—	0.95
August 4	21	4.8	—
11	22	4.8	—
10	17	3.4	1.30
25	22	2.4	1.50
September 9	25	4.2	2.50

TABLE 6. Water chemistry data for Haynes Lake. Total phosphorus and chlorophyll *a* were collected in the epilimnion (0–4 m).

Date	Total phosphorus (μg/L)	Chlorophyll *a* (μg/L)	Secchi depth (m)
April 16	—	6.5	2.6
25	20	5.5	3.6
29	17	2.6	3.8
May 5	15	0.8	4.8
13	15	0.8	5.2
20	17	0.3	4.5
27	11	0.2	6.2
June 3	14	0.3	4.0
9	14	1.7	3.9
17	13	0.6	4.1
24	12	0.7	3.9
July 1	19	1.2	3.4
8	18	3.0	2.0
15	—	—	3.2
22	18	0.3	4.2
29	14	5.7	4.4
August 5	13	1.3	4.4
12	—	—	4.5
19	15	1.9	4.5
26	16	0.9	4.0
September 7	—	1.0	5.1

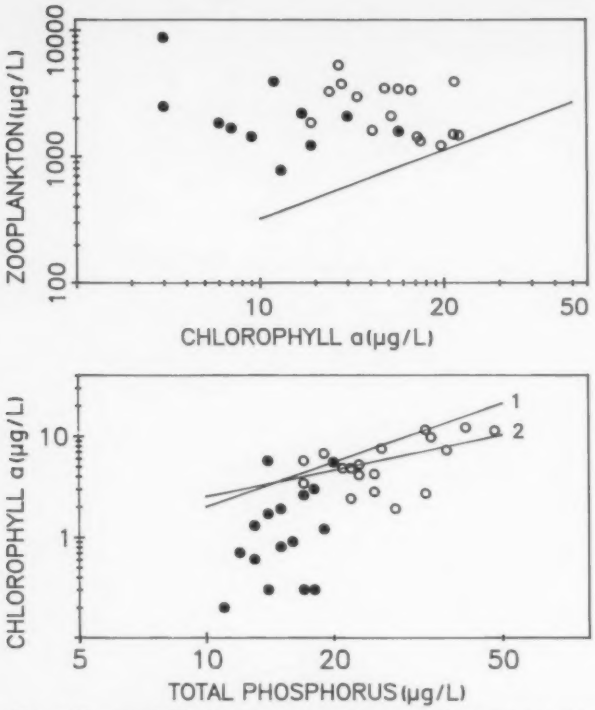

FIG. 4. Top panel: Weekly zooplankton biomasses (fresh weight) with respect to chlorophyll *a* at Lake St. George (open circles) and Haynes Lake (solid circles). The line is a general regression relationship for zooplankton biomass versus chlorophyll *a* based on 82 lake-years of data (McQueen et al. 1986). Bottom panel: Weekly concentrations of chlorophyll *a* versus epilimnetic phosphorus for Lake St. George (open circles) and Haynes Lake (solid circles). Line 1 is a regression based on 42 lake-years of data (Dillon and Rigler 1974). Line 2 is a regression based on 140 lake-years of data (Janus and Vollenweider 1981).

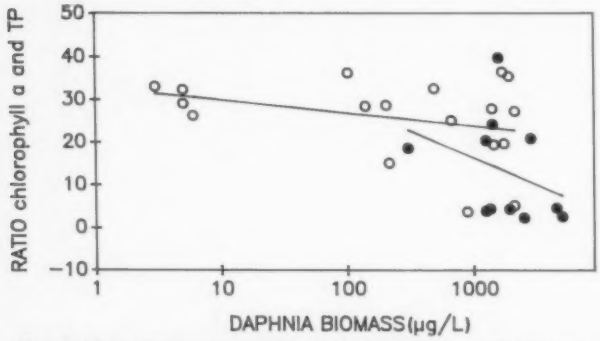

FIG. 5. Ratio (arcsine transformed) of chlorophyll *a* to total phosphorus (both µg/L) plotted with respect to *Daphnia* biomass. The upper regression line and open circles are for Lake St. George; the lower regression line and solid circles are for Haynes Lake.

toplankton biomass and water clarity than communities comprising smaller individuals and that they may have more effect (McQueen et al. 1986; Vanni et al. 1990) in oligotrophic lakes (Haynes) than in eutrophic lakes (St. George). Finally, that phytoplankton biomasses are always strongly related to nutrient availability and that in eutrophic systems, nutrients are likely to be the primary determinants of phytoplankton biomass and water clarity.

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Factors Governing Clutch Size in Two Species of *Diaptomus* (Copepoda: Calanoida)

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We conducted a synoptic survey of 11 nonstratifying lakes in eastern Quebec to investigate the relative importance of water temperature, food concentration ([Chl a]), female prosome length, copepod density (population and community densities), and mate availability (sex ratio and density of males) in determining the clutch size of two freshwater copepods, *Diaptomus minutus* and *D. oregonensis*. Multiple regression analyses indicated that 60% of the variation in clutch size of *D. minutus* could be accounted for by temperature and [Chl a] ($n = 33$; $P = 0.0001$), while 80% of the variation in the *D. oregonensis* data ($n = 11$; $P = 0.0017$) was explained by temperature and prosome length. Addition of other variables to either model did not significantly reduce the residual variation. We also investigated the effect of interspecific interaction on the egg-bearing potential of *D. minutus* and found some evidence that animals in sympatry may have lower clutch size than those in allopatry when temperature and food concentration were held constant. By using an independent measure of food and mate availability, we confirmed that clutch size decreased significantly in relation to food limitation for both species, but was unaffected by mate limitation for either.

Nous avons procédé à une étude synoptique de onze lacs de l'est du Québec non marqués par la stratification pour examiner l'importance relative de la température de l'eau, de la concentration d'aliments ([Chl a]), de la longueur du prosoma chez les femelles, de la densité des copépodes (densités des populations et des communautés) et de la disponibilité d'un compagnon ou d'une compagne (ratio de mâles à femelles et densité des mâles), dans la détermination de l'importance des groupes de deux copépodes d'eau douce, *Diaptomus minutus* et *D. oregonensis*. Les analyses de régression multiple ont indiqué que 60 % de la variation dans l'importance des groupes de *D. minutus* pouvaient s'expliquer par la température et la [Chl a] ($n = 33$; $P = 0{,}0001$), alors que 80 % de la variation observée dans les données relatives aux *D. oregonensis* ($n = 11$; $P = 0{,}0017$) s'expliquaient par la température et la longueur du prosoma. L'addition d'autres variables à un modèle ou l'autre ne réduisait pas pour la peine la variation résiduelle. Nous avons également examiné l'effet de l'interaction interspécifique sur le potentiel ovigère de *D. minutus* et constaté que les dimensions des groupes d'animaux en sympatrie pouvaient être moins importantes que celles des animaux en allopatrie, lorsque la température et la concentration des aliments étaient maintenues constantes. En recourant à un moyen indépendant de mesurer la disponibilité des aliments et des compagnons ou compagnes, nous avons confirmé le fait que les dimensions des groupes diminuaient considérablement avec la limitation des aliments pour les deux espèces, mais que les dimensions des groupes ne variaient pas avec la limitation des femelles ou des mâles pour aucune des espèces.

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The reproductive capacity of calanoid copepods may depend on several factors that include mating success, length of the oviducal cycle, clutch size, and egg mortality. Of these, more appears to be known about the effect of clutch size than any other variable, perhaps because it is relatively easy to measure on a routine basis. As a result of this level of interest, some generalities are beginning to emerge. For instance, some dramatic seasonal and site-to-site variations in clutch size within both freshwater and marine taxa have been correlated with changes in the body size of females (Smyly 1968; Maly 1973, 1983; Cooney and Gehrs 1980; Elmore 1983), copepod density (Maly 1973), temperature, food availability (Cooney and Gehrs 1980; Checkley 1980a, 1980b), and a combination of the last two (Elmore 1983; Williamson and Butler 1987).

With few exceptions, most investigations have been carried out in the laboratory where usually only one factor at a time was examined or where the species of interest was studied in the absence of potential competitive effects from other diaptomid species. Although there is a large accumulation of data from single-factor, single-species experiments, these results may have limited applicability to natural situations, where populations are exposed to a number of ecological factors, and where ubiquitous species tend to occur with many other species (e.g. *Diaptomus minutus*; Carter et al. 1980). Without some confirmatory field studies where all factors are considered simultaneously, it is difficult to evaluate the relative importance of laboratory-identified factors in determining diaptomid clutch size in lakes.

A method was recently developed by Williamson and Butler (1987) to evaluate the relative effect of food availability and mate density on egg production of *D. pallidus*. They formulated indices, based on quantifiable responses of the oviducal cycle

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to changes in food levels or mate density, to infer food or mate limitation. The food-limitation index (f-index) was calculated as $f = [N/(N+G+O+B)] \times 100$, where N = nongravid females, G = gravid females, with visibly darkened oocytes, O = ovigerous females, bearing external egg sacs, and B = both ovigerous and gravid. An f value of zero indicated that there was no food limitation, since all females were gravid, ovigerous, or both whereas an f value of 100 implied severe food limitation. The mate-limitation index (m-index) was calculated as $m = [G/(G+O+B)] \times 100$. Since diaptomids usually require a successful mating prior to each clutch extrusion. Williamson and Butler interpreted an m value of 100 to indicate extreme mate limitation, but a zero value to indicate no mate limitation, since none of the copepods would be gravid. These indices should be generally applicable to other copepods, provided that life history characteristics of the species examined are similar to those of *D. pallidus*.

In this paper, we investigate the simultaneous effects of surface water temperature, food availability, copepod density, female prosome length, mate availability, and species association on clutch-size variability of *D. minutus* and *D. oregonensis*, copepods commonly found in lakes of eastern North America (Carter et al. 1980). Data from a detailed field survey are used to determine the relative importance of these factors and to construct empirical models to predict diaptomid clutch size through multiple regression analyses. We also use the f- and m-indices as indirect measures of food and mate limitation to provide an independent means of validating the conclusions of the multiple regression analyses. By establishing the relative importance of the different environmental and density-dependent factors, we hope that investigators will concentrate on the more important variables in future studies, and eventually develop a unifying theory to describe the factors that govern reproductive success of *Diaptomus* in nature.

Methods and Materials

The detailed field survey was conducted in 1985 and involved five sampling trips to 11 lakes over a 3-mo period between mid-May and mid-August. On each visit, Secchi depth transparency was determined in duplicate, and water samples were collected for pH measurement. We also recorded the water temperature (T) just below the surface and near the sediment at the deepest station of each water body to determine the stratification characteristics on each sampling date.

Composite water samples were collected in triplicate from representative sites in the lake or pond for [Chl *a*] (CHL) (Strickland and Parsons 1972). Although CHL is only a crude estimate of food availability, since not all algal particles found in lakes are ingested by *D. minutus* and *D. oregonensis* (P. Chow-Fraser, unpubl.), it is necessarily the variable of choice in synoptic studies because of ease of measurement (e.g. Zimmerman et al. 1983; Pace 1984, 1986). It is also a useful index of trophic status (i.e. Carlson 1977) because of its significant correlation with total phosphorus concentrations (e.g. Dillon and Rigler 1974). In this study, we use CHL as a broad indicator of food availability, although size-fractionated biomass estimates would be more accurate indicators.

On each lake visit, 24–26 samples of zooplankton were collected at randomly determined positions or depths with 2-L Schindler-Patalas traps for estimates of diaptomid density (TD = total adult density of all species in lake, DDM = adult density of *D. minutus*, and DDO = *D. oregonensis*); approximately half of these were obtained at the top 1 m and half from the bottom 1 m of the lake column. Duplicate qualitative plankton samples were also obtained with a Wisconsin plankton townet from a boat or from shore. All zooplankton samples were preserved in 6% formalin in the field for later identification and enumeration in the laboratory. Because we omitted a prefixation step with chloroform–ethanol solution prior to formalin preservation, we may have artificially reduced the number of ovigerous females appearing in all our samples by up to 22% (n = 7) and 28% (n = 5) for *D. minutus* and *D. oregonensis*, respectively.

The clutch size (CS) and prosome length (L) of 20 females of each species from each of these samples were determined with the aid of a dissecting microscope equipped with stage and eye-piece micrometers; measurements were made to the nearest 0.03 mm. It was sometimes necessary to detach and dissect egg sacs to measure clutch size accurately. We determined mate availability as the male to female ratio (MF) of the first 100 adults encountered in qualitative samples for each species and estimated male densities (MD) using the formula $[(M/(M+F)) \times \mathrm{DD}]$ where M and F were the number of males and females, respectively, in the qualitative field subsample and DD was the corresponding copepod density estimated from Schindler-Patalas trap samples.

We extracted relevant information from the qualitative plankton samples to calculate Williamson and Butler's (1987) indices for *D. minutus* and *D. oregonensis* in our lakes. For each species on each lake date, we scored the number of females in the first 100 females encountered that were O, G, B or N, as indicated above, and followed appropriate formulae to calculate these indices (see the introduction). The reduction in the proportion of ovigerous females in our samples, due to omission of a prefixation step prior to formalin preservation, should not interfere with our statistical analyses because the bias occurred consistently in all our samples.

Results and Discussion

Field Survey

Diaptomus minutus occurred allopatrically in five of the 11 lakes sampled in the survey, sympatrically with *D. oregonensis* in four, and sympatrically with *D. birgei* in the remaining two (Table 1). The corresponding CHL and Secchi depth transparency values place all lakes in this study within the mesotrophic–oligotrophic range, except Choiniere, which falls within the eutrophic category (Environment Canada 1981). While in most cases the pH was circumneutral, a few of the lakes located on the Canadian Shield were mildly dystrophic and had slightly lower pH. The lakes were well mixed in most instances, except for Croche Lake which exhibited periodic weak thermal stratification during July. At no time did the temperature differ by more than 6°C between surface and bottom in any lake.

Because of the highly variable patterns of zooplankton population growth rates in these lakes, it was not always possible to obtain relevant information for all species on each lake visit. Therefore, data for only 45 of the 55 lake visits from the survey have been included for further statistical analyses, and these are presented in Tables 2 and 3 for *D. minutus* and *D. oregonensis*, respectively.

We carried out a correlation analysis to explore relationships between CS of *D. minutus* and T, L, CHL, DD, TD, MD, and

TABLE 1. Geographic location, species distribution (DM = *D. minutus*; DO = *D. oregonensis*; DB = *D. birgei*), maximum lake depth (m), mean summer Secchi depth transparency (m), mean summer (Chl *a*] (CHL, μg/L), and mean temperature differential between top and bottom waters during the season (*T*-Diff. °C) in study lakes. Mean values are based on 5–10 measurements.

Lake	Latitude	Longitude	Depth	Species found	Secchi	CHL	*T*-Diff
Thibault	45°58′40″	74°01′15″	2.5	DM, DO	1.4	6.9	0.6
Long	45°14′50″	72°19′45″	6.5	DM, DO	3.3	2.7	1.4
Pin Rouge	45°57′40″	74°02′30″	12.5	DM, DO	2.2	6.6	1.0
Cromwell	45°59′21″	73°59′50″	9.0	DM, DO	2.0	5.4	2.8
Roxton	45°28′00″	72°39′20″	6.0	DM, DB	1.8	6.8	1.4
Choiniere	45°25′10″	72°36′22″	14.5	DM, DB	1.0	18.8	1.0
Coeurs	45°58′07″	74°00′35″	7.1	DM	2.1	7.4	0.8
Croche Lake	45°59′21″	73°59′50″	10.5	DM	3.5	2.4	3.7
Croche Pond	45°59′21″	73°59′50″	3.3	DM	2.2	4.5	1.7
Sally	45°10′45″	72°26′20″	2.0	DM	2.2	2.3	1.4
Libby	45°16′35″	72°22′00″	3.5	DM	2.0	5.3	1.6

MF. (The latter was first arcsin transformed but all others were log transformed.) CS was significantly correlated with all variables except MD and MF (Table 4). Although L was significantly correlated with CS, we eliminated it from further analysis because of strong covariance between L and CHL ($r = 0.67$, $P = 0.001$).

The remaining variables were regressed against CS in two stepwise regression analyses; in one, we excluded TD and in the other we excluded DD. After CHL and T were entered into the respective regression models, addition of DD or TD did not explain any significant amount of the remaining residual variation; therefore, both were dropped from subsequent analysis. We then regressed CS against T and CHL and obtained the following regression equation:

$$(1)\quad \log CS = -1.18\,(\pm 0.347)\log T + 0.22(\pm 0.071)\log CHL + 2.192\quad P = 0.0001,\ r^2 = 0.60,\ n = 33$$

(numbers in parentheses are the SE). This model explained 60% of the variation in the dependent variable and indicated that clutch size of *D. minutus* was both a positive function of food concentration (Fig. 1a) and a negative function of water temperature (Fig. 1b). The interaction term did not account for any significant amount of the residual variation (slope = −0.01; $P = 0.99$) even though there was a significant negative correlation between T and CHL ($r = 0.53$; $P < 0.05$).

The strong individual effects of temperature and food concentration have been well documented for several species of *Diaptomus*. Elmore (1983) showed that a temperature decrease from 30 to 20°C produced up to a twofold increase in CS for three subtropical species. Similarly, we found a twofold increase in CS when temperatures decreased from 25 to 20°C when food concentration was held constant (Eq. 1). The strong effect of CHL noted in this study is also consistent with that reported for *D. clavipes* (Cooney and Gehrs 1980), *D. mississipiensis*, *D. floridanus*, *D. dorsalis* (Elmore 1983), and *D. pallidus* (Williamson and Butler 1987). Although each of these two factors had an independent effect on CS, there is a suggestion in our study that both factors act in combination to regulate CS in field populations. This additive effect of temperature and food, however, is unlike the interactive effect noted by Williamson and Butler (1987) who found that starvation effects were more pronounced at high than at low temperatures in their laboratory trials.

To examine the effect of species association (i.e. allopatric versus sympatric occurrences) on clutch size of *D. minutus*, we sorted the entire dataset according to lakes in which *D. minutus* occurred allopatrically and those in which they occurred sympatrically with *D. oregonensis* or *D. birgei*. We then repeated the regression analysis on the sorted dataset. This procedure increased the r^2 value slightly to 0.64 and 0.69 for allopatric and sympatric lakes, respectively, and yielded the following two equations:

Allopatric:

$$(2)\quad \log CS = -1.11\,(\pm 0.429)\log T + 0.26\,(\pm 0.087)\log CHL + 2.139\quad P = 0.0005,\ r^2 = 0.64,\ N = 18.$$

Sympatric:

$$(3)\quad \log CS = -1.17\,(\pm 0.535)\log T + 0.27\,(\pm 0.118)\log CHL + 2.092\quad P = 0.0009,\ r^2 = 0.69,\ n = 15.$$

The slightly improved r^2 value as well as numerically higher regression coefficients for T and CHL in Eq. 2 and 3 compared with Eq. 1 suggest that the effect of food concentration on clutch size should be studied separately for allopatric and sympatric populations of *D. minutus*. Although the coefficients in Eq. 2 and 3 were not statistically different (Tukey Kramer test, $P > 0.05$), the intercept associated with allopatric populations was numerically higher. This suggests that when T and CHL are held constant, the CS of allopatric copepods are higher compared with sympatric individuals. This is consistent with the observation that on average, CHL associated with allopatric populations are only 56% of the values associated with sympatric populations for essentially the same CS (Table 5).

The lower than expected CS in sympatric populations may mean that interspecific interaction (competition for food) has an adverse effect on the egg-bearing potential of *D. minutus* when it cooccurs with *D. birgei* or *D. oregonensis*, perhaps because both congeners are larger. For instance, the dry weight of *D. oregonensis* is more than twice that of *D. minutus* (3.84–8.66 compared with 1.97–3.55 μg, respectively; Culver et al. 1985) and this disparity in biomass may alone account for the difference in CS per unit CHL between allopatric and sympatric populations of *D. minutus*. Thus, the effect of food on clutch size may operate differently for species in allopatry than those in sympatry when size disparity is involved.

A similar correlation analysis was performed on *D. oregonensis* data (Table 6). In this instance, T, L, and TD were the only significant correlates of CS. A stepwise regression analysis revealed that after T and L were entered, addition of TD did not significantly improve the original r^2 value in the model. We then regressed CS against T and L to obtain the following multiple regression equation:

TABLE 2. Clutch size (CS), female prosome length (L, mm), [Chl a] (CHL, μg/L), surface water temperature (T, °C), adult diaptomid density (DDM, no./2 L), and male to female ratio (MF) for *D. minutus*.

Lake	Sampling date	CS	L	CHL	T	DDM	MF
Thibault	14 June	6.7	0.76	9.5	15	6.48	1.04
	5 July	5.4	0.80	7.8	24	3.58	1.00
	26 July	5.0	0.77	4.9	22	4.28	0.75
	19 August	5.0	0.80	5.4	20	0.38	0.24
Long	17 May	10.1	0.76	4.3	14	1.44	0.45
	7 June	6.4	0.72	3.4	17	3.56	0.96
	2 July	6.8	0.71	4.1	21	10.41	0.59
	23 July	2.6	0.66	1.2	23	6.41	0.55
	13 August	2.9	0.67	0.7	24	16.12	0.72
Pin Rouge	21 May	15.1	0.82	11.8	14	1.50	0.92
	14 June	4.6	0.80	11.6	16	0.62	0.67
	19 August	—	0.75	1.1	20	0.12	0.20
Coeurs	21 May	—	0.88	7.2	14	0.21	0.31
	14 June	18.6	0.92	12.8	16	0.76	0.79
	5 July	9.0	0.90	9.6	23	5.24	0.43
	26 July	4.9	0.86	2.6	22	1.52	0.39
Croche Lake	16 May	11.2	0.72	2.4	14	3.22	0.85
	11 June	6.4	0.75	5.4	16	0.68	1.22
	4 July	5.1	0.73	2.8	19	0.92	0.22
	25 July	3.7	0.74	0.4	22	0.46	0.18
	14 August	4.6	0.72	1.1	22	0.92	1.63
Sally	17 May	12.8	0.81	3.0	16	1.84	0.84
	7 June	6.1	0.77	3.9	17	3.96	0.59
	2 July	3.6	0.75	2.2	24	15.83	0.96
	23 July	4.7	0.73	1.7	22	17.11	0.49
	13 August	4.7	0.77	0.8	24	11.63	0.50
Libby	17 May	10.0	0.75	6.8	15	0.04	—
	7 June	8.4	0.77	7.7	17	0.46	0.89
	2 July	4.8	0.76	4.0	22	2.48	1.23
	23 July	8.0	0.77	5.9	22	0.52	2.19
	13 August	9.1	0.76	3.7	23	1.81	0.69
Roxton	6 June	8.4	0.80	10.1	15	3.35	—
	19 July	5.4	0.73	2.3	23	1.00	—
Choiniere	6 June	21.2	0.94	29.2	15	0.17	—
	19 July	5.0	0.76	8.2	22	0.68	—
Cromwell	16 May	—	0.79	9.5	18	0.36	0.31
Croche Pond	11 June	—	0.74	8.8	18	1.20	2.03
	4 July	—	0.74	6.3	22	0.10	2.19
	25 July	—	0.74	1.3	23	3.72	1.00
	14 August	—	0.76	4.9	22	1.82	1.19

$$(4)\quad \log CS = -1.681\ (\pm 0.406)\log T + 4.177\ (\pm 1.183)\log L + 3.175\ P = 0.0017,\ r^2 = 0.80,\ n = 11.$$

The CS of *D. oregonensis* thus appears to be both a positive function of L (Fig. 2a) and a negative function of T (Fig. 2b); but in contrast with *D. minutus*, does not appear to be related to CHL.

Mate and Food Limitation Indices

The regression of clutch size against the *f*-index confirmed the significant effect of food availability on clutch size for *D. minutus* (Fig. 3a). Clutch size decreased as a function of the *f*-index, indicating that clutch size became reduced with decreased food availability. This indirect relationship between CHL and the *f*-index suggests that Chl *a* is an adequate indicator of food availability for this species. Similarly, there was a significant regression between clutch size of *D. oregonensis* and the *f*-index (Fig. 4a). This, however, deviates from conclusions reached in the earlier section, in which we found no significant correlation between CS and CHL (Table 6). This discrepancy probably stems from the inappropriateness of using CHL as a measure of ambient food availability for *D. oregonensis*, especially because *D. oregonensis* appears to have a more specialized diet than does *D. minutus* (P. Chow-Fraser, unpubl. data). This comparison suggests that food may have emerged as an important determinant of clutch size in the multiple regression analysis (Eq. 4) if measurements of food availability were based on cell counts of edible food items rather than on CHL. The

TABLE 3. Summary data for *D. oregonensis*. Abbreviations and units are the same as in Table 2 except that DDO = adult diaptomid density of *D. oregonensis*.

Lake	Sampling date	CS	*L*	CHL	*T*	DDO	MF
Thibault	14 June	—	1.03	9.5	15	—	0.57
	5 July	9.9	1.04	7.8	24	9.90	0.59
	26 July	10.6	1.06	4.9	22	10.60	0.75
	19 August	8.4	1.06	5.4	20	8.35	0.56
Long	17 May	20.3	1.03	4.3	14	0.68	1.08
	7 June	—	1.11	3.4	17	0.12	1.85
	2 July	4.4	0.88	4.1	21	1.48	0.41
	23 July	—	—	1.2	23	1.13	0.67
	13 August	6.0	0.98	0.7	24	0.68	0.50
Cromwell	11 June	—	1.01	9.52	18	0.32	0.67
	4 July	9.4	1.02	6.23	20	1.38	0.72
	25 July	—	1.04	2.83	22	1.22	0.51
	14 August	8.4	1.01	2.7	24	0.64	0.41
Pin Rouge	14 June	14.7	0.97	11.6	16	0.46	0.37
	4 July	10.2	0.97	4.6	22	2.27	0.31
	26 July	—	—	3.7	21	0.38	0.42
	19 August	14.8	1.08	1.1	20	0.37	1.44

TABLE 4. Summary of correlations between clutch size of *D. minutus* and other variables of interest. See Table 2 for explanation of abbreviations.

n	Variable	*r*	*P*
33	*L*	+0.655	0.0001
33	CHL	+0.670	0.0001
33	*T*	−0.687	0.0001
33	DDM	−0.406	0.0190
33	TD	−0.364	0.0374
29	MF	−0.086	0.6905
29	MD	−0.219	0.2627

lack of a significant relationship between clutch size and the *m*-index also validates the conclusion made earlier that mate availability is not an important predictor of brood size for either species (Fig. 3b and 4b).

General Discussion

We have evaluated the relative importance of temperature, food concentration, prosome length, copepod density, and mate availability on the clutch size of two diaptomid species in 11 shallow, generally well-mixed lakes and ponds. Surface water temperature emerged as a strong negative correlate of clutch size for both *D. minutus* and *D. oregonensis* in our lakes (Eq. 1 and 4; Fig. 1b and 2b). This strong correlation was evident despite our use of surface water temperature, even in lakes that exhibit weak thermal stratification during the open-water season. There was also a very strong positive effect of food concentration for *D. minutus* (Fig. 1a) which was confirmed by the significant regression between CS and the *f*-index (Fig. 3a). Although CS of *D. oregonensis* was not significantly correlated with CHL, there was nevertheless a significant regression between clutch size and the *f*-index (Fig. 4a). Therefore, if more lakes had been sampled or if size-fractionated CHL data had been available, food concentration may also have emerged as a significant predictor of clutch size for this species.

Clutch size of both species was also positively correlated with female prosome length (Fig. 2b) even though the strong

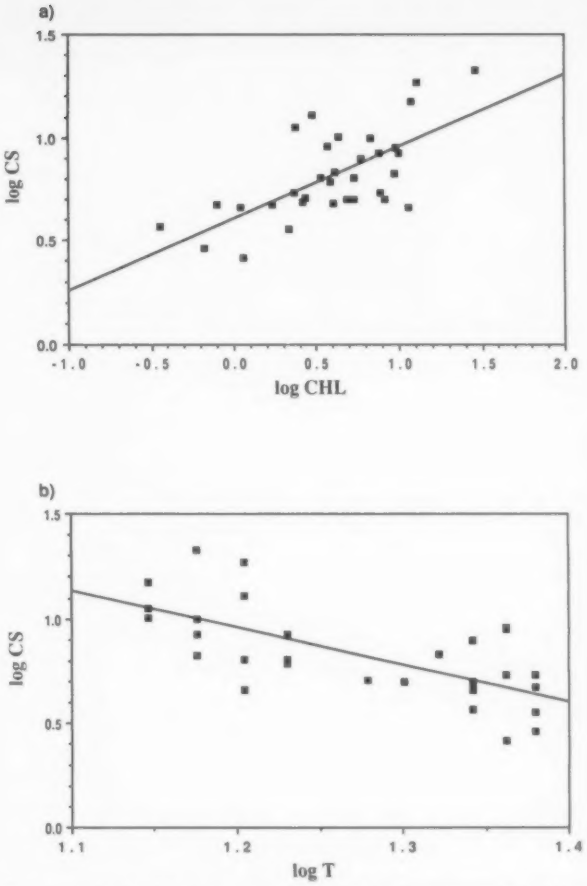

FIG. 1. (a) Log clutch size versus log CHL for *D. minutus*. Equation of the least-squares regression line is log CS = 0.3408 log CHL + 0.6076; $r = 0.67$, $P = 0.0001$, $n = 33$. (b) Log clutch size versus log temperature for *D. minutus*. Equation of the least-squares regression line is log CS = −1.7916 log *T* + 3.1102; $r = 0.69$, $P = 0.0001$, $n = 33$.

TABLE 5. Comparison of mean (SE in parentheses) clutch size (CS) with variables of interest for allopatric and sympatric *D. minutus* populations. See Table 2 for explanation of abbreviations. *Means are significantly different ($P < 0.05$).

Variable	Allopatric	Sympatric
CS	7.536 (0.911)	7.373 (1.265)
L	0.78 (0.014)	0.77 (0.018)
CHL	4.3 (0.77)	7.6 (1.80)*
T	19.8 (0.81)	19.0 (1.01)
DDM	3.86 (1.256)	3.99 (1.140)

covariance between prosome length and food concentration for *D. minutus* eclipses the importance of length as a predictor of clutch size. Of much less importance were copepod density (either population density or total diaptomid density) and mate availability (sex ratio or male density), neither of which had any significant effect on clutch size once the other three variables had been considered. Absence of a significant regression between clutch size and the *m*-index confirms that changes in clutch size for either species are unrelated to mate density.

TABLE 6. Summary of correlations between clutch size of *D. oregonensis* and other variables of interest. See Table 2 for explanation of abbreviations.

n	Variable	*r*	*P*
11	*L*	+0.603	0.0495
11	CHL	+0.215	0.5250
11	*T*	−0.695	0.0176
11	DDO	−0.337	0.3108
11	TD	−0.643	0.0328
11	MF	−0.107	0.8015
11	MD	−0.207	0.5421

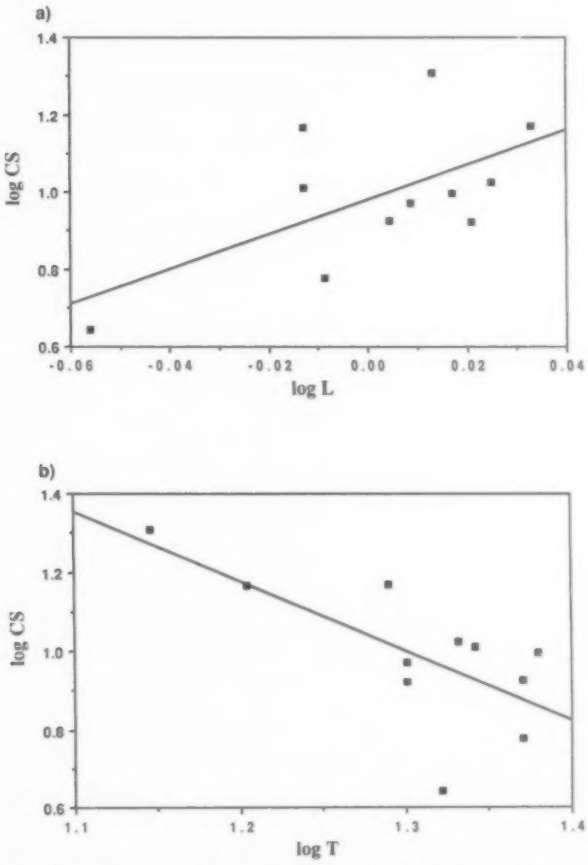

FIG. 2. (a) Log clutch size versus log length for *D. oregonensis*. Equation of the least-squares regression line is log CS = 4.5191 log *L* + 0.9794; $r = 0.61$, $P < 0.05$, $n = 11$. (b) Log clutch size versus log temperature for *D. oregonensis*. Equation of the least-squares regression line is log CS = −1.7689 log *T* + 3.3015; $r = 0.70$, $P = 0.02$, $n = 11$.

The effects of temperature on egg production have been studied in several freshwater and marine calanoid copepods, with inconsistent findings. Whereas Checkley (1980b) and Elmore (1983) both observed a decrease in clutch size with increased temperature for *Paracalanus parvus* and three subtropical freshwater diaptomids, respectively, Williamson and Butler (1987) found essentially no change in clutch size of *D. pallidus* at 25 compared with 15°C (17.5 and 16.8, respectively). In our study, we also noted an inverse relationship between clutch size

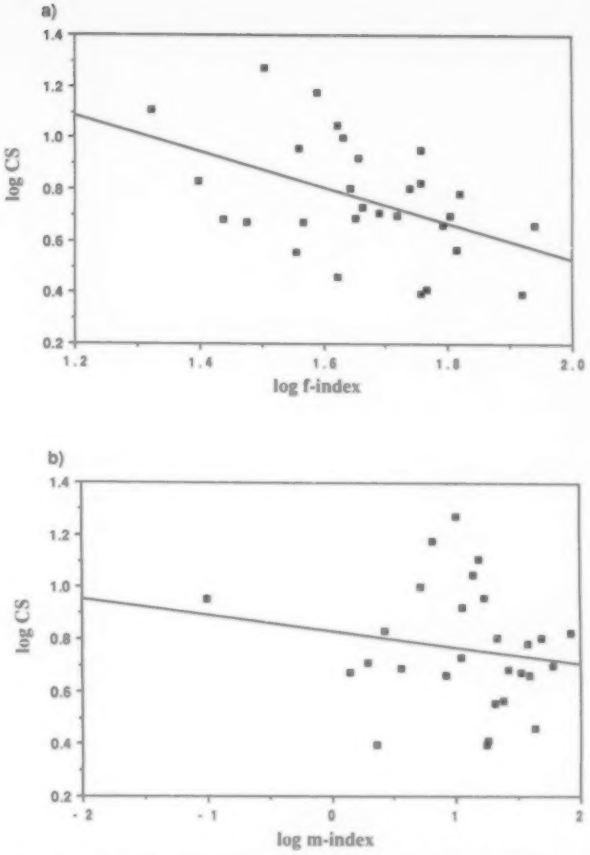

FIG. 3. (a) Log clutch size versus log *f*-index for *D. minutus*. Equation of the least-squares regression line is log CS = −0.6955 log *f*-index + 1.9202; $r = 0.45$, $P = 0.02$, $n = 33$. (b) Log clutch size versus log *m*-index for *D. minutus*. Equation of the least-squares regression line is log CS = −0.0607 log *m*-index + 0.8303; $r = 0.17$, $P = 0.40$, $n = 29$.

and temperature for *D. minutus* and *D. oregonensis*; however, our results differ from earlier studies with respect to the mechanism responsible for lower brood size. Both Checkley and Elmore attributed the reduction in clutch size to the smaller size of females with increased temperature; by comparison, in this study the clutch size of *D. minutus* was clearly more strongly influenced by food level than by body length, indicating that body length is not the primary determinant of clutch size for *D. minutus*. Therefore, the effect of temperature on clutch size in natural populations may vary from species to species, and generalities cannot be made regarding the genus without further research on more species.

Another factor that deserves attention is the effect of temperature on interclutch duration; although Williamson and Butler (1987) did not observe any temperature effects on clutch size of *D. pallidus*, overall egg production was increased because of significantly shorter interclutch duration at the higher temperature. Thus, even if warmer temperatures should bring about reduced clutch size in other species, the associated increase in egg development rate and rate of clutch production may more than compensate for the smaller brood size. This may have important implications to lake ecosystem research, especially in light of the anticipated global changes in weather pat-

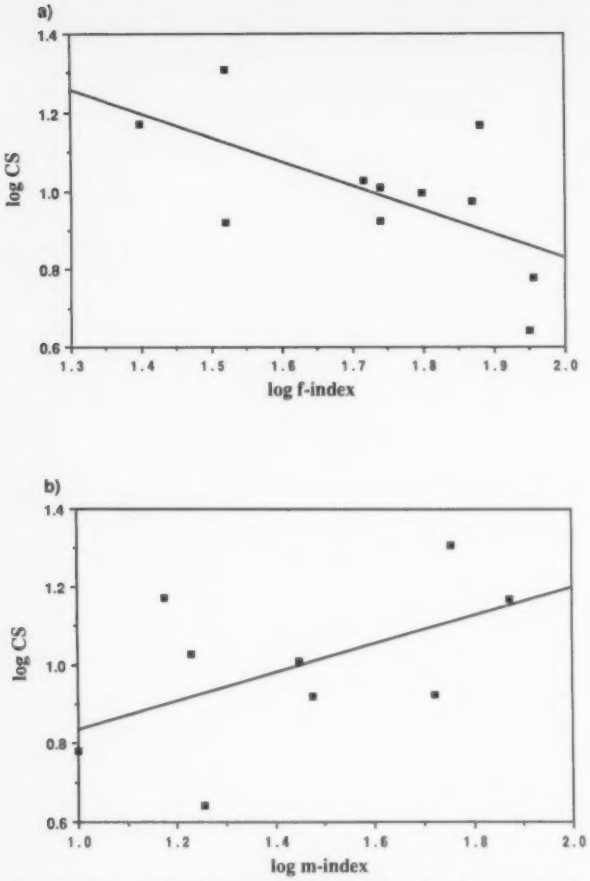

FIG. 4. (a) Log clutch size versus log *f*-index for *D. oregonensis*. Equation of the least-squares regression line is log CS = −0.6158 log *f*-index + 2.0605; $r = 0.62$, $P = 0.04$, $n = 11$. (b) Log clutch size versus log *m*-index for *D. oregonensis*. Equation of the least-squares regression line is log CS = 0.363 log *m*-index + 0.472; $r = 0.53$, $P = 0.15$, $n = 9$.

terns over the next several decades. Future investigations should focus on understanding the mechanism by which temperature influences the various phases of the oviducal cycle and how these interact with food availability to regulate the reproductive capacity of diaptomids in lakes.

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Concentration and Distribution of PCB Congeners in Isolated Ontario Lakes Contaminated by Atmospheric Deposition

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Macdonald, C. R., and C. D. Metcalfe. 1991. Concentration and distribution of PCB congeners in isolated Ontario lakes contaminated by atmospheric deposition. Can. J. Fish. Aquat. Sci. 48: 371–381.

The concentration of 19 PCB congeners was analysed in biota, sediments, water, and suspended solids in four central Ontario lakes in which atmospheric deposition was the major source of PCB contamination. Input from the atmosphere resulted in total congener concentrations of 1–2 $ng \cdot L^{-1}$ dissolved in water, 10–50 $\mu g \cdot kg^{-1}$ (dry weight) in sediment, 5–10 $\mu g \cdot kg^{-1}$ (wet weight) in biota from lower trophic levels (i.e. zooplankton, golden shiner (*Notemigonus crysoleucas*)), and 10–30 $\mu g \cdot kg^{-1}$ (wet weight) in fish from upper trophic levels (yellow perch (*Perca flavescens*), smallmouth bass (*Micropterus dolomieui*)). The dominant PCB congeners in the lakes were the trichlorobiphenyl congeners 31(28) and the hexachlorobiphenyl congeners 153 and 138, consistent with congener distributions reported for vapour- and particulate-bound PCBs in the atmosphere. Discriminant analysis indicated slight differences in congener patterns between the study lakes, but the general pattern for isolated lakes was substantially different from point-source contaminated lakes, primarily due to the high proportion of congeners 31(28). There was no significant difference in the total PCB concentrations in biota (lipid basis) between lakes, but within the lakes, total PCB concentrations were significantly higher in yellow perch than in biota from lower trophic levels.

La concentration de 19 congénères des BPC a été analysée dans le biote, dans les sédiments, dans l'eau et dans les solides en suspension de quatre lacs du centre de l'Ontario où le dépôt de polluants atmosphériques est la principale source de contamination par les BPC. L'apport de polluants atmosphériques a mené à des concentrations totales de congénères de 1 à 2 ng·L:s-[1] dissous dans l'eau, de 10 à 50 $\mu g \cdot kg^{-1}$ (poids sec) dans les sédiments, de 5 à 10 $\mu g \cdot kg^{-1}$ (poids humide) dans le biote des niveaux trophiques peu élevés (zooplancton, chatte de l'est (*Notemigonus crysoleucas*), par exemple) et de 10 à 30 $\mu g \cdot kg^{-1}$ (poids humide) dans le poisson des niveaux trophiques supérieurs (perchaude (*Perca flavescens*), achigan à petite bouche (*Micropterus dolomieui*)). Les principaux congénères des BPC dans les lacs étaient les congénères 31(28) du trichlorobiphényle et les congénères 153 et 138 de l'hexachlorobiphényle, ce qui correspondait aux distributions des congénères signalées pour les BPC liés aux vapeurs et aux particules dans l'atmosphère. L'analyse discriminante indiquait de légères différences dans les profils des congénères entre les lacs étudiés, mais le profil général des lacs isolés était assez différent de celui des lacs contaminés par une source ponctuelle, principalement à cause de la forte proportion de congénères 31(28). Il n'y avait pas de différence appréciable dans les concentrations totales de BPC du biote (lipides) entre les lacs; cependant, dans les lacs, les concentrations totales de BPC étaient considérablement plus fortes dans la perchaude que dans le biote des niveaux trophiques inférieurs.

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The deposition of contaminants from the atmosphere into the aquatic environment has been well documented in the case of acidic deposition, but the extent and significance of the problem with regard to organic contaminants in general, or polychlorinated biphenyls (PCBs) specifically, are still unclear. The contribution of atmospheric deposition to total PCBs in the Great Lakes has been estimated to be as high as 90% (Lake Superior; Strachan and Eisenreich 1988) and is undoubtedly higher in isolated inland lakes (Swackhamer and Armstrong 1986; Johnson et al. 1988). Data from peat bog cores collected in the northern United States and central and eastern Canada have shown that the current atmospheric loading rates for PCBs are approximately 1.3–3.0 $\mu g \cdot m^{-2} \cdot yr^{-1}$ (Rapaport and Eisenreich 1988), values which closely correlate with levels determined from sedimentation rates in isolated lakes (Swackhamer and Armstrong 1986). The peat bog data also indicated that past atmospheric PCB concentrations were closely correlated with PCB production in the United States and, as a result, have shown a significant decline since restrictions were placed on the use of PCBs in the early 1970s (Rapaport and Eisenreich 1988).

Several studies, however, indicate that current rates of atmospheric deposition are high enough to permit accumulation of PCBs to substantial levels in fish. In Ontario, Johnson et al. (1988) reported mean PCB concentrations of 53.9 $\mu g \cdot kg^{-1}$ (wet weight) in northern pike (*Esox lucius*) taken from lakes on the Bruce Peninsula and concentrations of 66.3 $\mu g \cdot kg^{-1}$ (wet weight) in brook trout (*Salvelinus fontinalis*) taken from the Turkey Lakes region. In Siskiwit Lake, an isolated lake on Isle Royale in Lake Superior, PCBs in whitefish (*Coregonus clupeaformis neohantoniensus*) have reached concentrations as high as 3000 $\mu g \cdot kg$ lipid^{-1}, although current levels are probably lower (Swackhamer and Hites 1988).

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There is some evidence to suggest that the pattern of PCB congeners, and their distribution, in lakes in which the atmosphere is the major source of PCBs will be different from lakes receiving point-source inputs of PCBs. Although the bulk of PCBs exist in the vapour phase in the atmosphere (Duinker and Bouchertall 1989; Baker and Eisenreich 1990), deposition occurs primarily by particulate fallout (Swackhamer et al. 1988; Rapaport and Eisenreich 1988). The distribution and concentration of the PCBs in the two phases in the atmosphere are largely temperature dependent, and hence vary seasonally (Manchester-Neesvig and Andren 1989). Furthermore, input of PCBs to lakes probably involves a mechanism whereby PCBs are added to lakes in rain or snow, and then some congeners volatilize back to the atmosphere (Murphy 1984). Hence, unlike point-source contaminated lakes, the amount and type of PCBs entering isolated lakes are expected to vary seasonally and also depend on other variables such as the lake surface area and distance from the source of PCBs (Swackhamer et al. 1988). Volatilization and sedimentation, the two major processes removing PCBs from the water column, will also vary seasonally and depend on the temperature regimen and particulate load of the lake. The interaction of the environmental and limnological factors with the physical properties of the PCB congeners will define both the amount and specific set of congeners present in a lake system, and hence the amount available to the biota.

In a previous study, Macdonald and Metcalfe (1989) determined that the pattern of PCB congeners in the biota of a relatively pristine lake in Ontario (Lake Scugog) was different from the congener pattern in the same groups of biota from two lakes contaminated by known point-sources of PCBs. PCBs in Lake Scugog samples comprised primarily the same congeners that dominate PCBs in the particulate and vapour phases of the atmosphere (Duinker and Bouchertall 1989; Manchester-Neesvig and Andren 1989). However, the PCB congener pattern observed in Lake Scugog, and the variation in congener profiles among the groups of biota, may have been an artifact of the particular limnological and trophic characteristics of the lake.

In this study, we surveyed four other isolated lakes to (1) determine whether the PCB congener patterns consistently correspond to the PCBs found in the atmosphere and (2) define the PCB concentrations that can be expected in biotic and abiotic compartments of lakes receiving only atmospheric deposition. The four study lakes are located to the north and east of industrialized areas bordering Lake Ontario where atmospheric contaminants may originate. It is our hypothesis that there will be similar PCB concentrations in the biotic and abiotic compartments of all isolated lakes, and that the congener patterns will be unique to lakes receiving only atmospheric inputs. These data will help to define the extent of PCB atmospheric contamination in central Ontario lakes, and may also aid in determining whether lakes have been contaminated by additional point-sources of PCBs.

Materials and Methods

Study Lakes

Initial selection of the four study lakes was based on data from the Ontario Ministry of the Environment's Sport Fish Testing and Information Program, which reported total PCB concentrations for lake trout (*Salvelinus namaycush*) and smallmouth bass (*Micropterus dolomieui*) muscle to be below or near the detection limit of low-resolution gas chromatography (<20 ppb). Final selection of the lakes was based on the presence of common species of benthic invertebrates and fish species, including golden shiner (*Notemigonus crysoleucas*), bluntnose minnow (*Pimephales notatus*), yellow perch (*Perca flavescens*), smallmouth bass, and, where possible, lake trout.

The four study lakes, which are classified as oligotrophic, are situated in central Ontario, Canada (Fig. 1). Three of the lakes are surrounded by cottage property (Boshkung Lake, St. Nora Lake, Wood Lake), and there is little development around the fourth, Opeongo Lake, which is situated in Algonquin Provincial Park. The lakes are located between 120 and 220 km northeast of the major urban and industrial centers of western Lake Ontario. The study region experiences winds originating from the south and southwest at an annual frequency of between 20 and 30% (Environment Canada 1980). The location of Lake Scugog is included in Fig. 1 for reference, since the distribution of PCBs has been previously described for this inland lake (Macdonald and Metcalfe 1989). Morphometric and water chemistry data for the lakes are summarized in Table 1.

Sampling

All sampling of biota and sediments was conducted in August – November 1986 and May – July 1987. Yellow perch, smallmouth bass, and lake trout were caught with a 1.3- or 2.6-m trap net or by angling. Golden shiner and bluntnose minnow were collected using a 20-m bag seine. Where possible, approximately the same size of fish was collected for each species from each lake to minimize differences in PCB concentrations due to body size. Clams (*Elliptio complanata*) were collected using hand-held dip nets. Zooplankton were collected using a 0.25-m^2 conical net with 276-μm nytex mesh and a brass cod bucket. Tows were made for approximately 5 min at a depth of 5 – 10 m and the plankton collected in two or three tows grouped to give one sample. The plankton were further concentrated by passing the sample through 64-μm mesh. With the exception of the trap nets, all sampling equipment was soaked in reagent-grade methanol prior to use. Generally, samples of biota were collected in several areas of the lake to give a representative sample.

Total body weight and length of all fish were measured in the field before dissection and freezing. Samples were frozen in solvent-rinsed aluminum foil. A sample of muscle tissue was taken from the dorsal area of the fillet below the dorsal fin for lake trout, smallmouth bass, and yellow perch samples. With smaller fish (golden shiner and bluntnose minnow), the head, tail, and internal organs were removed before analysis. Clam samples consisted of soft tissue removed from the valves.

Sediment cores were collected from the deepest area of each lake using a K-B corer with brass tubes. After collection of the core, the upper 3 cm was removed with a solvent-washed aluminum sectioning device and stored in solvent-washed glass jars. Sediments which were analysed for total organic carbon were collected in the same area as those for residue analysis, using an Ekman grab. Sediment samples were analyzed for organic carbon content according to the methods described by the Ontario Ministry of the Environment (1983).

Water and suspended solids samples were collected and treated in the field as described by Macdonald and Metcalfe (1989). Briefly, water was pumped from a depth of approximately 1 m into a stainless steel container. Lake water (18 L) was passed through glass fibre filters (pore size of 0.3 μm) into

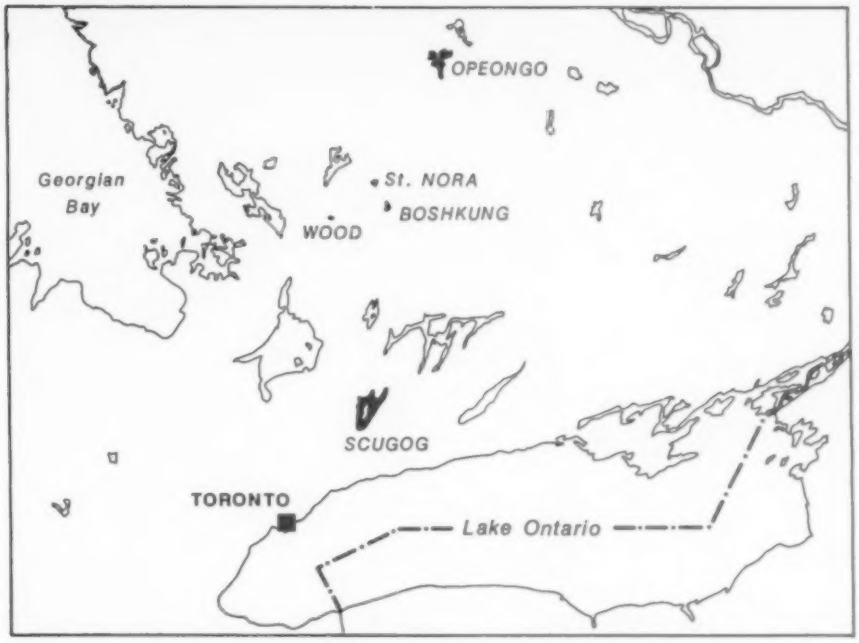

FIG. 1. Location of study lakes in central Ontario, Canada.

TABLE 1. Lake morphometry and water chemistry data for the four study lakes. Lake morphometry data are from the Ontario Ministry of the Environment (unpubl. data).

	Boshkung	Wood	St. Nora	Opeongo
Surface area (10^6 m^2)	7.2	1.5	2.6	58.6
Volume (10^6 m^3)	165	19	42	856
Mean depth (m)	23.0	5.0	16.0	14.6
Maximum depth (m)	68.0	14.0	39.0	49.4
pH	7.2	6.9	6.9	7.1
Alkalinity (mg $CaCO_3 \cdot L^{-1}$)	10.4	5.2	4.4	6.9
DOC (mg·L^{-1})	2.9	3.6	2.6	4.0
Suspended solids (mg·L^{-1})	0.9	1.1	1.0	1.0

a 20-L stainless steel extraction vessel. Extraction took place in the field by liquid/liquid partitioning into methylene chloride. Filters and associated suspended solids were placed in glass jars with approximately 100 mL of methylene chloride. Grab samples of water were also collected for gravimetric analysis of suspended solids.

Sample Preparation

Samples were prepared for high-resolution gas chromatographic analysis of PCB congeners using the methods described by Macdonald and Metcalfe (1989). Briefly, sediment samples of approximately 5 g were extracted with acetone by sonication and back-extracted into methylene chloride. The extract was dried by passing through sodium sulphate and rotary evaporated to 2 mL. Concentrated extracts were cleaned up and fractionated into three subfractions by column chromatography on activated silica gel. Any PCBs, DDE, aldrin, lindane, heptachlor, and mirex eluting in Fraction A were separated from other organochlorine pesticides eluting in Fractions B and C. Sulfur compounds were removed from Fraction A by precipitation with mercury.

Biota samples of approximately 5 g were ground with sodium sulphate and extracted into hexane on a Soxhlet apparatus for 1 h. Lipids were removed from the extract by gel permeation chromatography (GPC) on a column of Biobeads SX2. The GPC eluent containing lipid was collected and evaporated to dryness for gravimetric determination of lipid content. The eluent containing PCBs and organochlorine pesticides was collected and subjected to further clean-up and fractionation by silica gel column chromatography, as described above.

The methylene chloride collected in the field from two water samples (2 × 18 L) was pooled, passed through sodium sulphate, and partitioned into hexane. The pooled sample was then cleaned up and fractionated by silica gel chromatography, as described above. The glass fibre filters containing suspended particulate material were manually broken up while immersed in the methylene chloride originally added in the field. The entire sample was filtered under suction through sodium sulphate. The extract was partitioned into hexane and cleaned up/fractionated by silica gel chromatography, as described previously.

Sample Analysis

Extracts were analysed for PCBs by high-resolution gas chromatography on a Varian 3500 gas chromatograph with electron capture detector, using a 30-m DB5 capillary column and splitless injection. Gas chromatographic (GC) data were analysed with a Nelson Analytical PC integration system (Ver 5.0). The GC conditions were as described by Macdonald and Metcalfe (1989). Individual congeners were identified by retention time (window 0.02%) and quantified from integrated peak areas.

The 19 PCB congeners selected for analysis were (in order of elution from DB-5 column) congeners 18, 31(28), 52, 49,

44, 101, 87, 110(77), 151, 118(149), 153(132), 138, 180, 170, 201, 196, 195, 194, and 209. Quantification was by comparison with standards purchased from the National Research Council in Halifax, N.S., Canada, as described by Macdonald and Metcalfe (1989). As denoted by the parentheses (above), congeners 31, 110, 118, and 153 coelute with other PCB congeners. In these cases, the GC peak was quantified according to the response factor for the congener in the standard, which should represent the majority of the PCB found in environmental samples. However, since congeners 31 and 28 are found in approximately equal proportions in commercial Aroclors (Schulz et al. 1989), we assumed that this peak represents an equal mixture of the two congeners. The 19 congeners represent approximately 46, 53, and 62% of the total PCBs present in Aroclors 1242, 1254, and 1260, respectively (Schulz et al. 1989).

Extraction efficiencies for all quantified PCB congeners were determined by analyses of spiked samples to be >85% for biota and sediment samples and >75% for water and suspended solids sampled. Soxhlet extraction of biota samples for 4 h did not improve the extraction efficiency, in comparison with the 1-h protocol used routinely in this study. Data are reported without correction for extraction efficiencies. Sample blanks were run at intervals of approximately every eight samples. Replicate analysis of muscle tissue ($n = 5$) from each of three perch and three smallmouth bass and from three sediment samples indicate that between-replicate variability was less than 10%.

Since peaks with integrated areas of >1000 could be detected over background noise ("instrumental detection limit"), the GC data system was set to ignore all peaks with areas <1000. The detector responded linearly over the range of PCB concentrations in the sample extracts. The "limit of detection" (LOD) was calculated according to Keith et al. (1983) as three times the standard deviation of mean concentrations of each congener detected in sample blanks. The LODs for the 19 congeners were between 0.3 and 1.0 $ng \cdot mL^{-1}$. Peak identification, congener concentrations, and the efficiency of the silica gel fractionation procedure were confirmed by GC/MSD analysis of selected samples.

Data Treatment and Statistical Analysis

The concentration of each congener in biota was calculated on a wet and lipid weight basis. The concentration of each congener in sediment and suspended solids was calculated on a dry weight basis and, in the case of sediment samples, on an organic carbon basis as well. The total PCB concentration was calculated as the sum of the 19 congeners analysed. The proportion of each congener was calculated as the concentration of the congener divided by the total.

Data were analysed using SPSS/PC Ver 2.0 statistical software (SPSS/PC, Inc., Chicago, IL) on a personal computer. All means and range tests were calculated using log-transformed data to equalize variances and normalize residuals (i.e. geometric means). Comparison of means was conducted using Scheffé's range test. Significant differences were measured at $p < 0.05$. Zero values (i.e. below detection limits) were replaced by random numbers between zero and the detection limit for discriminate analysis of the proportionate data (Sharaf et al. 1986). Zooplankton from Boshkung Lake were analysed on a dry weight basis, and lipid weight and wet weight congener concentrations were estimated by assuming that dry weight is 25% of wet weight. The Boshkung zooplankton data were not included in range test comparisons of PCB levels.

All discriminant analysis was conducted on the relative proportion of the individual congeners in smallmouth bass, yellow perch, golden shiner, zooplankton, clams, and sediment; samples which were common to all study lakes. Congener 209 was removed from the analysis because of the large number of zero values.

Results

Total mean PCB congener concentrations (wet weight) in biota ranged from 3.56 $\mu g \cdot kg^{-1}$ in zooplankton from Wood Lake to a maximum of 87.6 $\mu g \cdot kg^{-1}$ in lake trout from Boshkung Lake (Table 2). PCB concentrations on a lipid weight basis were relatively consistent at approximately 500 – 4000 $\mu g \cdot kg^{-1}$ in all groups of biota. Congener concentrations in water were often near detection limits, but total PCB levels in water ranged from approximately 1 to 2 $ng \cdot L^{-1}$. In sediment, total PCB concentrations ranged from 12 to 54 $\mu g \cdot kg^{-1}$ on a dry weight basis and from 160 to 550 $\mu g \cdot kg^{-1}$ on an organic carbon basis. Data for suspended solids were available for only two lakes, but the PCB concentrations in suspended solids were higher than the corresponding concentrations in the bottom sediments for both Opeongo and Wood lakes.

One-way analysis of variance (ANOVA) showed that the body weights and lipid contents of biota collected from the study lakes were very uniform. With the exception of the clam samples, there were no significant differences in body weight between lakes for any of the classes of biota. For fish, this was consistent with the age data, which showed that the mean age of yellow perch from all lakes was approximately 3 yr and all smallmouth bass were approximately 4+ yr old. This uniformity was a result of sampling specific body sizes of each species within the lakes. Lipid levels in yellow perch, smallmouth bass, and clams showed no significant differences between lakes, but the lipid contents of golden shiner and zooplankton samples from Opeongo Lake were significantly greater than at least one other lake.

On a wet weight basis, PCB levels in smallmouth bass were significantly higher in St. Nora and Boshkung lakes than in Opeongo and Wood lakes. The clam samples from Boshkung Lake were significantly higher in PCB concentration than in St. Nora or Wood Lake. Both the golden shiner and sediment (dry weight) samples from Opeongo Lake contained significantly higher concentrations of PCBs than any of the other study lakes. However, no lake showed consistently higher concentrations of PCBs in all classes of samples. On a lipid basis, there were no significant differences in PCB concentration between any of the lakes for any group of biota, with the single exception of PCBs in golden shiners, which were higher in Wood Lake than in Opeongo Lake.

Within each lake, the relative ranking of biota according to wet weight PCB concentration varied considerably. For example, smallmouth bass contained the highest concentrations of PCBs in Boshkung Lake, but smallmouth bass in Opeongo Lake contained among the lowest concentrations of PCBs in the biota of that lake. However, ranking of PCB concentrations on a lipid weight basis revealed that adult yellow perch contained the highest concentrations of PCBs among biota in all lakes. This difference was statistically significant in comparisons with at least one other group of biota in each lake. Total lipid weight PCB concentrations in lake trout samples were not as high as

TABLE 2. Summarized data of body weights, lipid levels, and total PCB congener concentration (wet weight and lipid weight basis) for sediments, biota, water, and suspended solids. Unless otherwise noted, all concentrations are in $\mu g \cdot kg^{-1}$. Values for body weights and PCB concentrations are geometric means whereas those for lipid weights are arithmetic. Values in parentheses are 1 SD about the mean. ns = not sampled because species not present.

	Boshkung	Wood	St. Nora	Opeongo
Abiotic				
Water				
Sample size (*n*)	2	2	2	2
PCB concentration ($ng \cdot L^{-1}$)	0.93 (0.82–1.05)	1.85 (0.86–3.99)	1.60 (1.51–1.69)	1.23 (0.99–1.52)
Suspended solids				
Sample size (*n*)	—	2	—	2
PCB concentration (dry weight)	—	367 (335–402)	—	220 (176–274)
Sediment				
Organic carbon (%)	3.6	9.8	5.28	9.87
Sample size (*n*)	5	4	5	4
PCB concentration (dry weight)	27.2 (19.7–37.6)	15.2 (14.0–16.5)	12.0 (9.47–15.1)	53.9 (44.4–65.5)
PCB concentration (organic C basis)	356 (257–494)	156 (144–169)	227 (179–287)	546 (449–663)
Biotic				
Zooplankton				
Sample size (*n*)	2	5	1	4
Lipid (%)	1.97 (1.76–2.18)	0.35 (0.29–0.41)	0.28	0.80 (0.73–0.87)
PCB concentration (wet weight)	11.6[a] (10.3–13.1)	3.56 (3.00–4.23)	4.36	6.11 (5.18–7.22)
PCB concentration (lipid basis)	592 (589–596)	1030 (742–1430)	1550	766 (655–895)
Clams				
Sample size (*n*)	5	5	5	5
Body weight (g)	32.5 (29.5–35.9)	8.33 (5.24–13.2)	8.74 (6.67–11.4)	26.7 (25.1–28.3)
Lipid (%)	0.37 (0.23–0.51)	0.28 (0.23–0.33)	0.23 (0.15–0.32)	0.39 (0.34–0.44)
PCB concentration (wet weight)	8.16 (6.09–10.9)	4.63 (3.83–5.60)	3.57 (2.52–5.06)	6.32 (2.10–7.20)
PCB concentration (lipid basis)	2330 (1850–2930)	1670 (1400–1990)	1590 (832–3040)	1630 (1350–1950)
Golden shiner				
Sample size (*n*)	5	3	4	4
Body weight (g)	7.90 (4.55–13.7)	6.40 (4.18–9.81)	5.58 (4.51–6.91)	8.17 (7.95–8.38)
Lipid (%)	0.86 (0.73–0.99)	0.37 (0.33–0.41)	0.84 (0.43–1.25)	2.49 (2.24–2.73)
PCB concentration (wet weight)	5.44 (3.88–7.62)	4.25 (2.97–6.09)	5.20 (3.62–7.47)	11.9 (10.4–13.7)
PCB concentration (lipid basis)	642 (416–990)	1170 (810–1690)	683 (488–956)	482 (445–522)
Bluntnose minnow				
Sample size (*n*)	4	5	5	5
Body weight (g)	4.99 (3.11–8.01)	2.81 (1.96–4.05)	2.82 (1.58–5.04)	5.16 (2.83–9.43)
Lipid (%)	0.95[b] (0.44–1.47)	2.37[c] (0.00–4.74)	1.07 (0.82–1.32)	0.89 (0.80–0.99)
PCB concentration (wet weight)	9.78 (7.35–13.0)	6.24 (4.44–8.76)	10.4 (6.84–15.8)	7.96 (6.39–9.92)
PCB concentration (lipid basis)	1130 (670–1920)	446 (105–1890)	993 (676–1460)	893 (708–1130)

TABLE 2. (*Concluded*)

	Boshkung	Wood	St. Nora	Opeongo
Yellow perch				
Sample size (*n*)	5	5	5	5
Body weight (g)	43.2 (33.6–55.5)	72.8 (38.1–139)	59.4 (30.8–114)	80.6 (57.4–113)
Lipid (%)	0.27 (0.22–0.32)	0.26 (0.19–0.33)	0.28 (0.19–0.37)	0.16 (0.11–0.21)
PCB concentration (wet weight)	11.4 (5.66–23.0)	8.76 (5.73–13.4)	8.43 (4.55–15.6)	4.98 (3.47–7.16)
PCB concentration (lipid basis)	4260 (2140–8460)	3440 (1980–3680)	3140 (1800–5470)	3310 (2060–5310)
Smallmouth bass				
Sample size (*n*)	4	5	5	5
Body weight (g)	454 (197–1047)	492 (285–851)	319[c] (178–573)	153 (134–175)
Lipid (%)	1.57 (0.00–3.14)	0.53 (0.17–1.67)	1.31 (0.69–1.93)	0.23 (0.14–0.32)
PCB concentration (wet weight)	25.5 (17.5–37.2)	6.17 (4.54–8.39)	35.4 (18.8–66.6)	4.77 (4.14–5.49)
PCB concentration (lipid basis)	2420 (1560–3760)	1160 (490–2750)	2910 (1580–5360)	2200 (1610–3020)
Lake trout				
Sample size (*n*)	4	ns	2	5
Body weight (g)	1241 (625–2465)	ns	431 (298–623)	3060 (1824–5132)
Lipid (%)	4.61 (2.72–6.51)	ns	0.72 (0.55–0.88)	2.45 (1.64–3.27)
PCB concentration (wet weight)	87.6 (57.6–133)	ns	17.4 (11.0–27.3)	48.8 (20.7–115)
PCB concentration (lipid basis)	1550 (869–2780)	ns	2460 (1980–3050)	2100 (1180–3740)

[a]Wet weight concentration calculated from dry weight concentration, assuming a dry weight of 25% of total weight.
[b]Mean value based on sample size of 3.
[c]Mean value based on sample size of 4.

TABLE 3. Pooled values of lipid content and total congener concentration (wet weight and lipid weight basis) for biota common to the four study lakes. All concentration data are in $\mu g \cdot kg^{-1}$. Comparisons of means was conducted using Scheffé's range test. Sample groups with the same letter are not significantly different. Lake trout data were not included in range test comparisons of means (see text).

Taxa	Sample size	Lipid content (%)	PCB concentration		Comparison of means
			Wet weight	Lipid weight	
Golden shiner	16	1.17 (0.33–2.01)	6.25 (3.85–10.1)	679 (442–1040)	A
Bluntnose minnow	17	1.30 (0.04–2.57)	8.36 (5.81–12.0)	804 (350–1850)	A
Zooplankton	12	0.77 (0.16–1.37)	5.28 (3.32–8.39)	880 (621–1250)	A B
Clams	20	0.32 (0.21–0.43)	5.41 (3.63–8.04)	1780 (1230–2580)	B C
Smallmouth bass	19	0.96 (0.00–1.91)	12.3 (4.65–32.6)	2040 (1060–3950)	C D
Yellow perch	20	0.24 (0.16–0.32)	8.05 (4.48–14.5)	3510 (2050–6010)	D
Lake trout[a]	11	2.93 (1.03–4.81)	50.0 (21.4–117)	2130 (1450–3110)	

[a]Samples taken from Boshkung, St. Nora, and Opeongo lakes only.

in yellow perch and were significantly higher than only golden shiner, zooplankton, and bluntnose minnow in one lake, Opeongo.

These relationships were also tested using two-way ANOVA on the log-transformed total PCB (lipid weight) data of six species common to Boshkung, Wood, St. Nora, and Opeongo lakes. The analysis again indicated that there was no significant difference in the PCB concentrations of biota between the four lakes ($F_{3,80} = 1.24$, $p = 0.301$); however, within the lakes, species differences in PCB concentration were highly significant ($F_{5,80} = 23.3$, $p < 0.001$). In order to further define this relationship, PCB concentration data (lipid weight) from all lakes were pooled for each species (Table 3) and range tests conducted. The data for lake trout from three of the lakes are included in Table 3 for comparison, although the data were not included in the ANOVA or range tests. The range tests showed that total PCB concentrations (lipid basis) for yellow perch samples were significantly higher than for all other groups of biota, except smallmouth bass. PCB concentrations (lipid weight) in both smallmouth bass and clams were significantly higher than concentrations in golden shiner and bluntnose minnow, but not zooplankton.

Although there was some variability between lakes, the dominant congeners in biota and sediment samples were the trichlorobiphenyl congeners 31(28) and the hexachlorobiphenyl congeners 153 and 138 (Fig. 2). These congeners accounted for approximately 50% of the total PCBs within biota. Sediments showed a wider range of congeners in comparison with biota, with a relatively high proportion of the tetrachlorobiphenyl congener 52, and often the pentachlorobiphenyl congener 101.

In Opeongo and Wood lakes, the congener patterns in biota were similar (Fig. 2), and congeners 31(28), 153, and 138 accounted for approximately 40 – 50% of the total PCBs within each group. In essence, all biota showed approximately the same pattern within the two lakes, with some variations in the proportion of the dominant congeners. Conversely, the distribution of congeners was more variable in the biota of St. Nora and Boshkung lakes. For example, PCBs in clams, zooplankton, and yellow perch from Boshkung Lake comprised primarily the trichlorobiphenyls 31(28) and the tetrachlorobiphenyl 52, with significant amounts of congener 110, while smallmouth bass and sediments contained primarily the penta- and hexachlorobiphenyls 110, 118, 153, and 138. This variability in congener pattern was also observed in St. Nora Lake samples, although the trichlorobiphenyls comprised a much larger proportion of the total PCBs. In both Boshkung and St. Nora lakes, there was a shift in the upper trophic levels (i.e. yellow perch, smallmouth bass) to the more highly chlorinated penta- and hexachlorobiphenyls (Fig. 2). This variability in congener pattern between groups of biota, and the shift to moderately chlorinated congeners in higher trophic levels, was not observed in biota samples from Wood and Opeongo lakes (Fig. 2).

Discriminant analysis of the congener proportion data showed that the differences in congener pattern among the study lakes could be used to characterize the individual lakes (Fig. 3), despite the variation in congener patterns between trophic levels. Three discriminant functions successfully classified 96.3% of the samples from Opeongo Lake, 73.1% from Boshkung Lake, 60.0% from St. Nora Lake, and 59.3% from Wood Lake. Overall for the four lakes, 72.4% of the samples were properly classified with the three discriminant functions. The first discriminant function accounted for 71% of the total variance and grouped Boshkung and St. Nora lakes on the basis of high proportions of congeners 110, 196, 118, and 87 (in descending order of correlation) and Opeongo and Wood lakes on the basis of higher levels of congeners 138 and 153 (Fig. 3). The second function accounted for only 16.5% of the total variance and separated Boshkung and Wood lakes on the basis of the proportion of the trichlorobiphenyls 31(28) and St. Nora and Opeongo lakes by the proportion of congeners 49, 151, and 195.

Discriminant analysis also showed that the proportions of congeners present in the sediment and biota samples from the remote inland lakes are markedly different from the proportions in samples from two other lakes (Lake Clear, Rice Lake) which have received inputs of commercial PCB Aroclors in the past (Ferguson and Metcalfe 1989; Macdonald and Metcalfe 1989). The congener distribution in lakes receiving atmospheric PCBs was clearly separated with two discriminant functions from lakes contaminated with Aroclor 1254 (Lake Clear) or with a mixture of Aroclors 1242, 1254, and 1260 (Rice Lake) (Fig. 4). The first discriminant function (78% of total variability) separated the two remote lakes from the contaminated lakes, primarily by the high proportion of congeners 31(28) in the remote lakes. The second function (15% of total variability) separated Rice Lake from the other lakes on the basis of their lower relative proportion of congeners 138, 153, 180, and 170. In total, over 95% of the samples were properly classified with the two discriminant functions according to their origin in one of the remote lakes, or Lake Clear, or Rice Lake.

Discussion

The goal of this study was to determine the pattern and concentration of PCBs in the biota, water, and sediments of inland lakes in Ontario in which PCB contamination could be directly attributed to atmospheric input. The congener pattern in the study lakes, particularly Opeongo and Wood lakes, was dominated by congeners 31(28), 153, and 138 and remained relatively consistent throughout the biota. The clear separation of Opeongo and Wood lakes from lakes with known contamination using discriminate analysis (Fig. 4) suggests that the source of contamination was the same for the two lakes. Furthermore, the relatively simplistic congener pattern suggests that the atmosphere was the sole source of contamination to the lakes and that the pattern is probably typical of isolated, oligotrophic lakes in central Ontario. This conclusion supports the view of Johnson et al. (1988) who reported that 90% of the PCBs entering isolated lakes in Ontario come directly from the atmosphere (with the remainder from the watershed). In addition, the high levels of 153 and 138 relative to the other congeners correlate well with the major PCB congeners observed in biota in the Canadian Arctic, a region which presumably receives PCBs only by atmospheric flux (Norstrom et al. 1988).

Further evidence for this is demonstrated in the close similarity between PCB concentrations and patterns in three of the study lakes and in Lake Scugog (Macdonald and Metcalfe 1989), another inland lake to the southwest of the study lakes (Fig. 1). Indeed, congener patterns in biota from Lake Scugog and St. Nora Lake are almost identical. Lake Scugog is a shallow (mean depth = 1.4 m), eutrophic lake with a large surface area (82.6×10^6 m^2) and no known point-sources of PCBs. Thus, even when comparing lakes with very different trophic

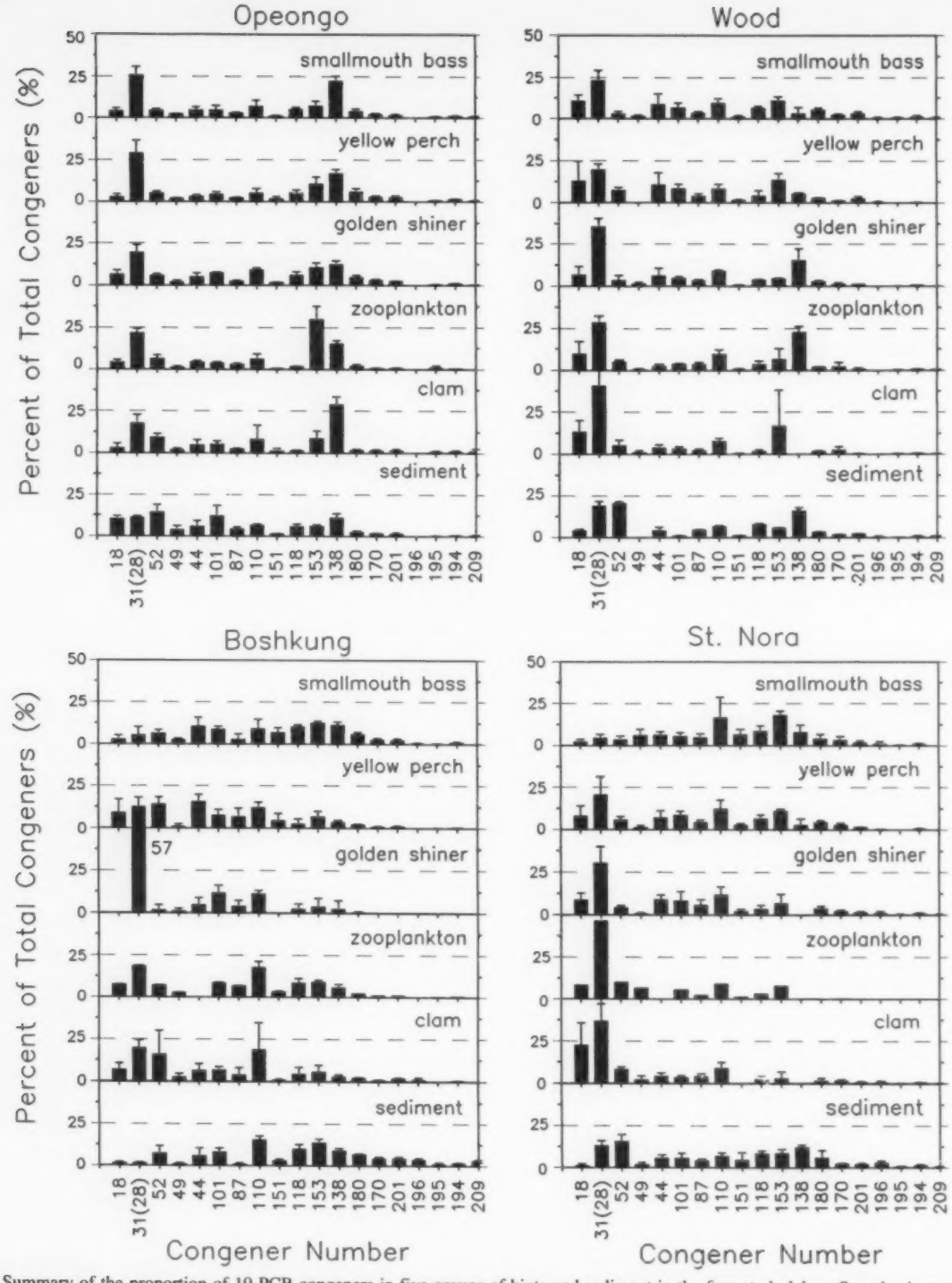

FIG. 2. Summary of the proportion of 19 PCB congeners in five groups of biota and sediment in the four study lakes. Sample sizes are given in Table 2. Mean value of congener 31(28) in Boshkung Lake golden shiner is 57% (SD = 8.02). For clarity, coeluting congener numbers have been removed from congeners 153, 118, and 110.

status and morphometry, the basic PCB pattern reflecting atmospheric input is retained.

The exact pattern of congeners which are deposited from the atmosphere is still unclear. Although atmospheric PCBs exist predominantly in the vapour phase (Atlas et al. 1986; Duinker and Bouchertall 1989), deposition occurs primarily by wet and

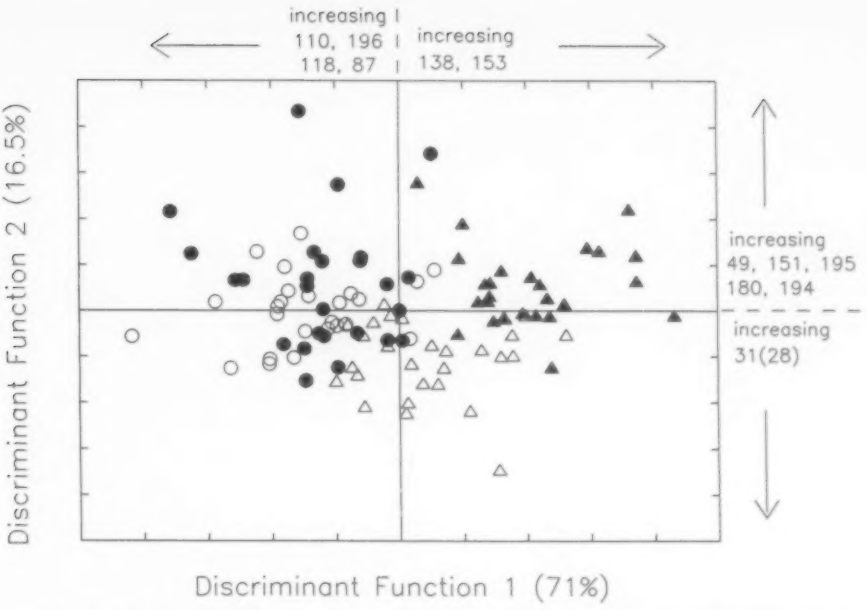

FIG. 3. Discriminant analysis comparison of the four study lakes using the congener patterns for the sample groups shown in Fig. 2. Numbers on axes indicate congener numbers with high correlations with the discriminant functions. ○, Boshkung Lake; ●, St. Nora Lake; Δ, Wood Lake; ▲, Opeongo Lake.

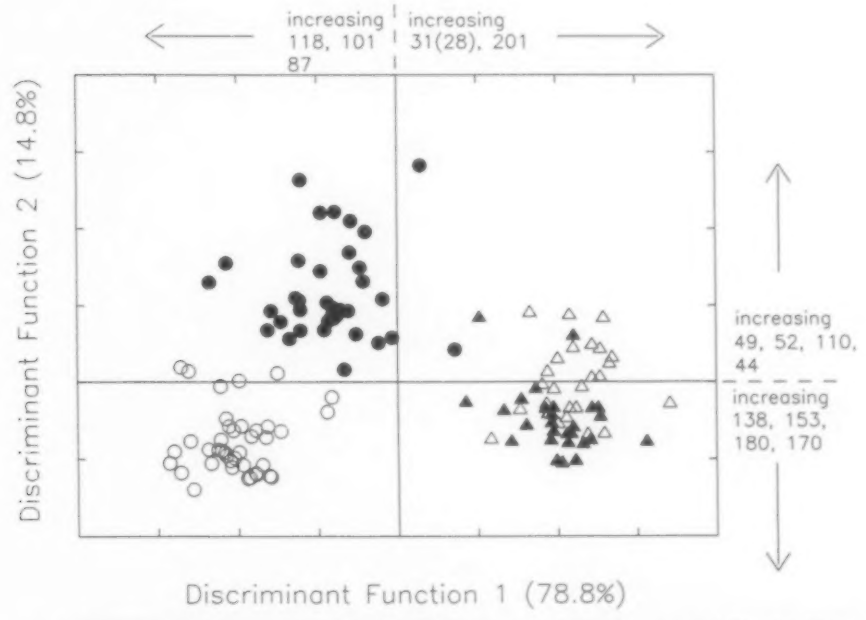

FIG. 4. Comparison of lakes contaminated by atmospheric deposition (Opeongo and Wood) with two lakes of known point-source contamination (see text). Numbers along axes indicate PCB congeners with high correlations with the discriminant functions. ○, Lake Clear; ●, Rice Lake; Δ, Wood Lake; ▲, Opeongo Lake.

dry fallout of particulates (Swackhamer et al. 1988). However, the distribution of congeners between the two phases in the atmosphere with respect to degree of chlorine substitution has not been resolved. Evidence suggests that the vapour phase is dominated by trichlorobiphenyls, which include congeners 31 and 28 (Duinker and Bouchertall 1989; Baker and Eisenreich 1990), while the particulate fraction is enriched with higher chlorinated congeners relative to the gas phase (Swackhamer

et al. 1988; Duinker and Bouchertall 1989; Baker and Eisenreich 1990). Predominance of penta- and hexachlorobiphenyls in precipitation has also been shown in peat bog cores (Rapaport and Eisenreich 1988), directly in precipitation (Murphy and Rzeszutko 1977), and in air samples (Eisenreich et al. 1981). Other studies have indicated that there is little difference in the congener profiles in the two phases and that both are dominated by lower chlorinated congeners (Manchester-Neesvig and Andren 1989). The high proportion of the trichlorobiphenyls 31(28) throughout the lakes in the present study suggests that washout of the lower chlorinated congeners from the vapour phase is occurring, or that there is a significant component of lower chlorinated congeners on the particulate fraction. Scavenging of the more water-soluble congeners from the vapour phase has been suggested by Swackhamer et al. (1988) as an explanation for the high concentrations of trichlorobiphenyls in rain samples at Siskiwit Lake.

Several other factors may affect the amount and type of PCBs deposited to a lake. Temperature has a significant influence on vapour–particulate partitioning and volatilization, and it has been shown that the flux of PCBs is substantially reduced in the winter (Swackhamer et al. 1988; Manchester-Neesvig and Andren 1989). Furthermore, Manchester-Neesvig and Andren (1989) reported seasonal differences in the distribution of specific congeners between the gas and particulate phases. Although there was no indication that there were major differences in congener distribution between the samples collected in the fall and spring in the present study, it is reasonable to assume that seasonal changes in the temperature of the air and surface water, in addition to ice cover for a large part of the year, have significant influences on PCB flux in the study lakes.

Geographic location may also play an important role in defining congener composition in lakes. Swackhamer et al. (1988) suggested that particle-bound PCBs may be lost as the distance increases between the source and the site of deposition, causing changes in the PCB composition within the air mass. The similarity in dominant congeners in Opeongo, St. Nora, and Wood lakes suggests that the composition of atmospheric PCBs is relatively homogeneous within the study area. However, the conclusions from this study should be applied to other geographical regions with caution.

The congener distribution in Boshkung Lake was more difficult to interpret in terms of atmospheric deposition. Although the proportion of congener 31(28) was high in golden shiner, all other groups of biota showed a wide range of congeners, which include the tri-, tetra-, penta-, and hexachlorobiphenyls, with no particular homolog group dominating. There are two possible explanations for this. First, Boshkung Lake is situated at the confluence of three major watersheds. The lack of a distinct pattern in Boshkung Lake may be due to a large watershed area, where deposition, volatilization, and sedimentation of the PCBs occurred in the individual lakes prior to flowing into Boshkung Lake. A second possibility is that there was an additional source of PCBs (e.g. contaminated road oil) in the Boshkung Lake watershed. The higher proportions of tetra- to hexachlorobiphenyls relative to congeners 153 and 138, and in particular the pentachlorobiphenyl 110, suggest that small amounts of a PCB mixture such as Aroclor 1254 may have been released into the watershed, although the amount would be relatively low. In addition, higher concentrations of total PCBs in some groups of biota (zooplankton, smallmouth bass, clams) in comparison with the other study lakes lend support to the possibility of inputs from additional sources.

Atmospheric deposition results in PCB concentrations of approximately 10–50 $\mu g \cdot kg^{-1}$ (dry weight) in sediments, 1–2 $ng \cdot L^{-1}$ in water, 5–10 $\mu g \cdot kg^{-1}$ (wet weight) in the lower trophic levels, and 10–30 $\mu g \cdot kg^{-1}$ in the upper trophic levels for the 19 congeners analysed here. If it is assumed that these 19 congeners comprise about 50% of Aroclor mixtures, then the total PCBs in biota would be up to approximately 20 $\mu g \cdot kg^{-1}$ (wet weight) in the lower trophic levels and up to 60 $\mu g \cdot kg^{-1}$ in the upper trophic levels. These values correspond well with PCB concentrations of 35–66 $\mu g \cdot kg^{-1}$ (wet weight) reported for four species of upper trophic level fish in two Ontario watersheds (Johnson et al. 1988). Similarly, the concentration of approximately 3000 $\mu g \cdot kg^{-1}$ (lipid) in whitefish in Siskiwit Lake and Lake Superior (Swackhamer and Hites 1988), two systems contaminated principally from the atmosphere, agrees with the values of 2040–3150 $\mu g \cdot kg^{-1}$ (lipid) reported here for yellow perch, smallmouth bass, and lake trout. Hence, although input from the atmosphere represents a significant source of contamination to isolated lakes, the concentration of PCBs in the upper trophic levels is unlikely to exceed 100 $\mu g \cdot kg^{-1}$ on a wet weight basis and 4000 $\mu g \cdot kg^{-1}$ on a lipid weight basis.

Despite substantial differences in surface area (from 1.5×10^6 to 58.6×10^6 m^2) and mean depth (5.0–23 m) between the four study lakes, and presumably the amount of PCBs deposited from the atmosphere, there are no consistent differences between the lakes in total PCB concentration in biota, particularly on a lipid weight basis. It can be concluded from this that the concentration of PCBs to which biota are exposed is roughly the same in each lake regardless of higher inputs to the lakes with larger surface areas. This would suggest that the higher inputs are balanced by higher losses through volatilization or transport to the sediment. Swackhamer et al. (1988) estimated that the losses of PCBs through these two processes were approximately equal in Siskiwit Lake. Sedimentation of PCBs may be an important process in Opeongo Lake, where the concentration of PCBs in the highly organic sediment (9.9% organic carbon) is significantly higher than in the other lakes, even though the concentration in biota is not significantly higher. The role of lake morphometry has also been demonstrated by Macdonald and Metcalfe (1989), who showed that the rate of recovery in the biota of shallow, PCB-contaminated lakes may be slowed by sediment resuspension.

Comparisons of total PCB concentration on a lipid weight basis within the lakes indicate that yellow perch, and possibly smallmouth bass and lake trout, contain higher concentrations of total PCBs (lipid basis) than zooplankton, clams, bluntnose minnow, and golden shiner. Several studies have shown that PCB accumulation in fish involves uptake through the gills and the gut, both of which are influenced by the metabolic rate, growth rate, exposure levels, and size of the fish (Norstrom et al. 1976; Thomann 1981, 1989; Jensen et al. 1982; Thomann and Connolly 1984). PCB accumulation is also affected by the period of exposure or age of the organism (van der Oost et al. 1988). Recently, it has been proposed that for compounds such as PCBs which have log octanol–water partition coefficients of 5–8, food chain transfer is the most significant method of exposure to the upper trophic levels (Connolly and Pedersen 1988; Thomann 1989). Hence, the higher levels observed in yellow perch, smallmouth bass, and lake trout are probably the result of several factors which are related to their elevated position in the food chain.

In summary, the congener patterns observed in at least three of the four isolated lakes in this study can be attributed to atmospheric deposition. Using discriminant analysis, it is possible to recognize those lakes receiving atmospheric input of PCBs from lakes which have received point-source discharges of commercial PCB mixtures. Finally, contamination of lakes by atmospheric deposition alone is not likely to produce wet weight PCB concentrations in fish flesh above 100 $\mu g \cdot kg^{-1}$, even for lipid-rich fish in higher trophic levels (i.e. salmonids).

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Concentrations of Inorganic Elements and Fatty Acids in Geographic Populations of the Spot Prawn *Pandalus platyceros*

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Inorganic elements and fatty acids were quantified in body segments of different size classes of the spot prawn *Pandalus platyceros* collected from various locations in British Columbia. Substantial differences in concentration of elements occurred in different body segments of the spot prawn, with the exoskeleton accumulating the highest number of quantifiable elements. Comparison of elements in abdomen carapace and tissue from immature males, mature males, and females indicated no correlation between element concentration and size, age, or sex of the spot prawn. Fatty acid profiles were similar for all body parts, except the hepatopancreas, and of the major fatty acids, only the content of 18:1*n*9 increased significantly with age of the spot prawn. Fatty acids and elements in the abdomen tissue of spot prawns from different locations were not significantly different and indicated similarity of diet irrespective of location. The concentrations of elements absorbed into the carapace of female spot prawns from separate geographic locations were substantially different, suggesting incorporation from elements in the ambient seawater, rather than uptake from diet, or any genetic variability associated with element metabolism in the spot prawn. The levels obtained for metallic elements in the spot prawn provide a measure against which future environmental impact could be assessed.

Des éléments inorganiques et des acides gras ont été évalués dans des segments d'organisme chez la crevette *Pandalus platyceros* de différentes catégories de dimensions, recueillie à divers endroits de la Colombie-Britannique. Il existait des différences considérables dans la concentration d'éléments, dans différents segments de l'organisme de la crevette, l'exosquelette accumulant le nombre le plus élevé d'éléments quantifiables. La comparaison des éléments présents dans la carapace abdominale et dans les tissus des mâles matures et immatures ainsi que des femelles n'a indiqué aucune corrélation entre la concentration des éléments et les dimensions, l'âge ou le sexe de la crevette. Les profils des acides gras étaient semblables pour toutes les parties de l'organisme sauf pour l'hépatopancréas; pour les principaux acides gras, seule la concentration de 18:1*n*9 augmentait sensiblement avec l'âge de la crevette. Les éléments et les acides gras des tissus abdominaux des crevettes provenant de divers endroits ne variaient pas pour la peine et révélaient un régime alimentaire semblable, peu importe l'endroit. Les concentrations des éléments absorbés dans la carapace des femelles en provenance de divers endroits étaient considérablement différentes, ce qui semble indiquer une assimilation d'éléments contenus dans l'eau de mer ambiante plutôt qu'une absorption d'éléments du régime alimentaire ou une différence génétique associée au métabolisme des éléments chez la crevette. Les concentrations d'éléments métalliques chez la crevette constituent un point de référence par rapport auquel on pourrait évaluer les incidences futures sur l'environnement.

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Marine invertebrates are known to accumulate inorganic elements from surrounding seawater and as such are effective monitors of metal concentrations in a particular habitat (White and Rainbow 1985). Coastal areas are subject to more environmental fluctuations than open oceans, and marine organisms have been used to monitor any elemental changes in ambient seawater (Bryan et al. 1980; Eisler 1981).

Calcium is the major element in the exoskeleton of crustaceans, and in freshwater species it is absorbed at premolt from the eroding exoskeleton and stored in the hepatopancreas for use during the next molt (Fieber and Lutz 1985). In marine species, no premolt storage of elements is required, as the calcium lost in the carapace during ecdysis is replaced readily by absorption from seawater (Greenaway 1983). Bioaccumulation of calcium and other elements from ambient seawater and element metabolism in crustaceans is not fully understood but could be genetically influenced. Thus the use of trace element analysis for stock identification of fish has been attempted (Behrens-Yamada and Mulligan 1982; Mulligan et al. 1983; Edmonds et al. 1989).

Studies on multielement accumulation in crustaceans have focused on mesopelagic decapods, and no information is available on larger pandalid shrimps (White and Rainbow 1987; Ridout et al. 1989). The baseline levels of elements in organisms are an important measure against which metal pollution can be assessed (Bryan 1968; Phillips 1976).

Type and abundance of diet, which are influenced by environmental conditions, are also a source of elements for bottom-dwelling detrital feeders. Fatty acid composition of lipids of marine animals generally reflect those of lipids in the prey organisms or diet, particularly the polyunsaturated fatty acids (PUFA's), since their rate of biosynthesis in animals is low (Ackman and Eaton 1967; Linko et al. 1985; Klungsoyr et al. 1989; Whyte et al. 1989). Fatty acid composition can therefore provide quantifiable markers of the diet being consumed at different stages in the organism's development and markers for prey or diet at specific habitats.

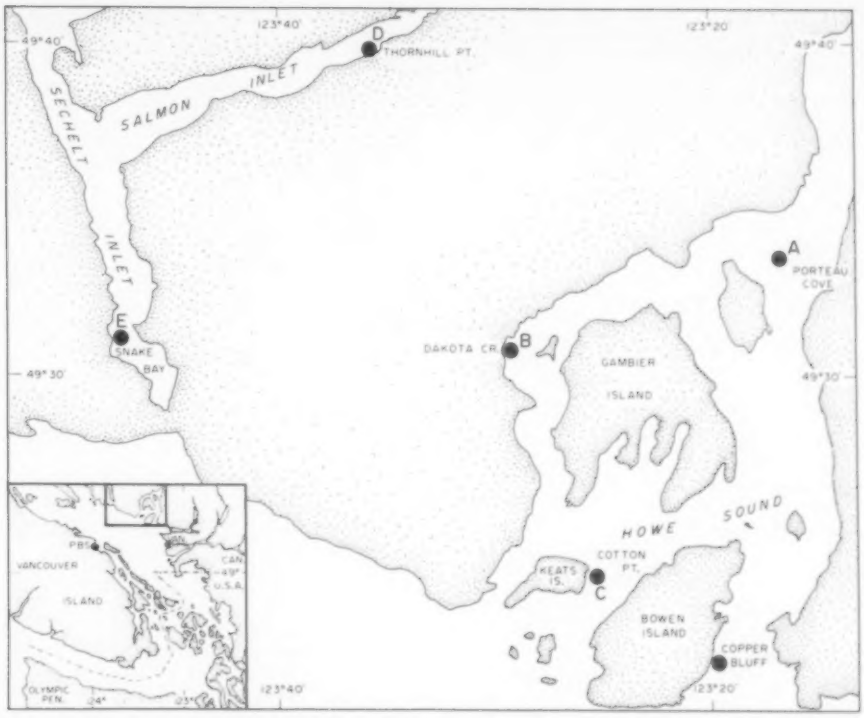

FIG. 1. Map of the area of the British Columbia coastline showing locations where prawns were collected.

The concentrations of inorganic elements and fatty acids in body parts of the spot prawn *Pandalus platyceros*, collected at different stages of development and from different sites, were assessed in this study to determine (1) what elements were actively accumulated by the parent and newly hatched spot prawn, (2) the most useful size class and body segment of a spot prawn suitable for elemental "database" analysis, (3) the potential of elemental analysis for stock identification, (4) the degree of uniformity of lipid constituents in the body of the spot prawn with age, (5) any dietary differences at collection sites based on fatty acid profiles, and (6) any relationship between diet and element accumulation.

Materials and Methods

To compare elemental composition of newly hatched larvae with parent spot prawns, female spot prawns carrying eggs were collected from Copper Bluff on the east side of Bowen Island (Fig. 1) in March 1988, returned live to the Pacific Biological Station, and held in filtered seawater (30 ppt, 9°C) without food for 28 d. Hatched larvae were collected daily, washed on a Nitex screen with cold 3.2% aqueous ammonium formate, and then freeze-dried and stored under vacuum at −80°C. A composite sample of larvae was prepared for elemental analysis.

To compare elements in different body parts, post-hatch females (28) from Copper Bluff were sectioned into abdominal carapace, abdominal tissue, hepatopancreas, and remaining head. The separated body parts were combined, freeze-dried, and then ground and vacuum sealed in oxygen barrier bags for storage at −80°C until analysed. A sample of the seawater used to hold the prawns was evaporated to dryness and the resultant salts analysed.

To investigate the potential of elemental analysis for stock identification, the degree of uniformity of lipid constituents, and any relationship between diet and element accumulation at different sites, spot prawns were collected from Porteau Cove, Dakota Creek, and Cotton Point in Howe Sound, Thornhill Point in Salmon Inlet, and Snake Bay in Sechelt Inlet (Fig. 1). Collections from each site were separated into categories according to stage of development. Immature males were rapidly growing age 0+ yr animals which would mature in the upcoming autumn. Mature males were mixed age 1+ and 2+ animals of which the 1+ yr class would function an additional year as males, while the 2+ yr class would undergo sex transition and function as mature females in the approaching autumn. Females, 3+ yr animals which had carried eggs on their pleopods from the previous autumn of 1987, had not molted for approximately 6 mo. Categorized specimens were frozen onboard and stored at −80°C until prepared for analysis.

Specimens (15) were partially thawed and separated into abdominal carapace, abdominal tissue, and remaining head including hepatopancreas. Eggs attached to females were removed from the pleopods and discarded. The combined spot prawn sections from each site were freeze-dried, ground, vacuum packed, and held at −80°C until analysed for elements and fatty acids.

Elemental composition was determined in a commercial laboratory using an inductively coupled plasma-source mass spectrometer (ICP-MS) scanning 68 elements at the parts per billion (ppb) sensitivity range and using indium and lutetium as internal standards. ICP-MS is used successfully for the analysis of

TABLE 1. Inorganic elements in body sections of post-hatch females and hatched larvae of *P. platyceros* from Copper Bluff (b.d. = below detectable level of 17 $ng \cdot g^{-1}$).

Elements (dry weight)	Abdomen carapace	Abdomen tissue	Hepatopancreas	Head	Larvae
Major ($mg \cdot g^{-1}$)					
Calcium[a]	62.57	31.26	5.66	62.67	7.25
Magnesium[a]	9.38	4.32	0.74	8.88	1.67
Sodium[a]	0.62	0.83	0.70	0.73	0.51
Strontium	1.07	0.36	0.01	0.95	0.00
Bromine	4.53	0.00	0.00	3.07	0.00
Minor ($\mu g \cdot g^{-1}$)					
Iron[a]	496.00	145.00	181.00	451.00	3.00
Boron	102.41	56.14	0.02	85.50	0.02
Titanium	56.44	46.79	0.02	55.14	0.02
Chromium[a]	3.28	2.13	1.35	3.14	1.31
Nickel[a]	3.30	2.03	0.93	2.77	0.51
Selenium[a]	20.60	7.19	6.89	14.70	0.78
Rubidium	2.91	6.98	4.97	3.67	5.65
Iodine[a]	150.92	0.02	0.02	82.48	0.02
Aluminum	20.52	11.58	15.90	38.67	8.23
Scandium	31.54	39.89	38.66	30.92	41.29
Manganese[a]	13.90	4.97	8.69	14.00	0.80
Copper[a]	83.10	92.30	534.60	99.40	27.40
Arsenic[a]	31.50	29.20	61.80	36.50	39.30
Barium	12.13	2.27	0.20	10.30	0.02
Zinc[a]	59.50	143.00	114.00	86.20	56.09
Silver	1.16	0.80	8.27	1.80	0.67
Cadmium	0.54	0.72	14.60	1.70	0.13
Palladium	6.00	1.48	0.02	5.64	0.02
Lithium	19.60	29.25	31.52	28.87	0.02
Trace ($ng \cdot g^{-1}$)					
Ruthenium	210	b.d.	b.d.	190	b.d.
Rhodium	114	55	b.d.	125	b.d.
Germanium	193	358	228	189	161
Yttrium	54	b.d.	36	47	b.d.
Dysprosium	b.d.	47	57	55	b.d.
Cobalt[a]	305	254	399	343	223
Vanadium[a]	332	1340	873	394	596
Lead	87	262	89	69	27
Tin[a]	8	27	29	10	9
Molybdenum[a]	249	407	1560	237	42
Tellurium	263	b.d.	b.d.	b.d.	b.d.
Lanthanum	46	b.d.	54	78	b.d.
Mercury	33	b.d.	101	54	b.d.
Hafnium	20	b.d.	b.d.	b.d.	b.d.
Gold	29	b.d.	b.d.	49	b.d.
Caesium	29	41	50	44	105
Uranium	55	b.d.	91	42	b.d.
Bismuth	18	65	54	b.d.	122
Thallium	22	b.d.	b.d.	21	b.d.
Antimony	178	83	212	65	173
Zirconium	73	105	166	b.d.	94
Samarium	31	68	136	44	b.d.
Niobium	57	b.d.	191	47	b.d.
Gadolinium	b.d.	124	b.d.	b.d.	b.d.
Tungsten	b.d.	b.d.	398	b.d.	b.d.

[a]Essential element.

marine biological reference materials (Ridout et al. 1988). Values quoted in the tables are only for those elements above the detection level, unless specific elements are higher and lower in certain tissues than the detection level and are recorded for comparative purposes.

Methyl esters of fatty acid components of total lipids in freeze-dried prawn segments were prepared by in situ saponification and methylation with methanolic boron trifluoride (Whyte 1988). Analyte solutions were separated on a Supelcowax 10 fused silica capillary column (30 m × 0.32 mm inside diameter, 0.25-μm film) maintained at 190°C for 32 min and then programmed to 240°C at 2°·min^{-1} and held at 240°C for 16 min. Helium carrier gas was controlled at 21 cm·s^{-1} with a split ratio of 100:1. Identification was made by comparison with standards and peaks assigned in accord with the data presented by Ackman (1986). Peaks of less than 0.2% of the total area were omitted from the profile. Fatty acid structure is represented as (L:BnX) where L = chain length, B = number of ethylenic bonds, and nX = the position of the double bond closest to the terminal methyl group in monomer or *cis* methylene-interrupted structures. The expression Σ4–6,*n*3/poly. represents the ratio of the sum of higher unsaturated fatty acids (HUFA's) with four to six double bonds in the *n*3 series of acids to the sum of polyethylenic acids. Single-factor ANOVA and Newman–Keuls multiple comparison tests on the different group means were performed on arcsine-transformed data (Zar 1984).

Results

A comparison of elements in larvae with those in parental tissue indicated an active accumulation of strontium, bromine,

TABLE 2. Inorganic elements in the abdominal carapace and tissue of immature male, mature male, and female *P. platyceros* from different locations (b.d. = below detectable level of 17 ng·g^{-1} for all elements except neodymium, europium, and lanthanum which were detected at 6 ng·g^{-1}).

	Thornhill Point						Snake Bay					
	Immature males		Mature males		Females		Immature males		Mature males		Females	
Elements (dry weight)	Carapace	Tissue	Carapace	Tissue	Carapace	Tissue	Carapace	Tissue	Carapace	Tissue	Carapace	Tissue
Major (mg·g^{-1})												
Calcium[a]	188.81	3.73	64.22	2.55	84.54	3.19	80.58	4.84	76.24	4.30	147.18	2.78
Magnesium[a]	14.88	2.63	16.42	1.61	23.00	2.16	16.69	2.67	17.00	2.56	18.60	2.07
Sodium[a]	4.37	0.47	1.76	0.27	1.81	0.44	1.92	0.49	1.77	0.62	4.23	0.55
Strontium	2.53	0.04	0.83	0.02	1.19	0.02	1.02	0.05	0.97	0.04	2.24	0.02
Iron[a]	0.27	0.05	0.94	0.07	1.27	0.06	1.19	0.06	1.11	0.07	0.14	0.06
Bromine	0.52	0.41	0.84	0.30	1.02	0.09	1.03	0.43	1.27	0.19	0.82	0.26
Minor (μg·g^{-1})												
Boron	66.20	8.71	35.36	4.43	75.07	1.85	29.63	0.12	33.03	0.14	120.19	0.12
Titanium	10.03	12.02	85.61	9.39	84.10	11.91	77.90	15.69	84.04	18.03	7.78	14.17
Chromium[a]	8.53	2.17	0.87	9.79	0.64	5.04	1.18	2.98	1.49	3.08	0.77	2.85
Nickel[a]	8.51	0.76	1.98	4.96	4.78	2.16	3.27	1.38	3.10	2.40	4.08	1.52
Selenium[a]	0.11	4.10	2.07	3.69	2.98	6.87	0.92	3.14	2.64	3.57	1.19	4.13
Rubidium	3.11	4.88	4.70	4.07	4.55	5.84	3.88	5.52	4.01	5.18	2.93	6.08
Iodine[a]	49.28	1.12	57.92	0.95	112.99	0.71	54.18	1.18	50.20	1.16	70.53	1.05
Aluminum	25.83	4.20	15.95	33.19	24.68	12.42	25.27	4.69	24.00	5.04	27.74	6.35
Scandium	3.66	3.74	97.91	2.60	97.99	5.11	82.08	6.55	90.40	8.09	3.89	4.88
Manganese[a]	35.72	4.01	14.31	3.29	25.32	3.80	17.00	4.04	21.06	4.72	59.92	3.21
Copper[a]	30.21	19.97	181.12	18.21	49.17	21.70	39.07	21.59	54.98	23.55	41.62	29.09
Arsenic[a]	9.78	23.19	17.82	26.95	33.27	79.93	13.37	16.53	17.38	30.49	23.74	48.71
Barium	9.86	0.15	2.88	0.31	4.89	0.15	2.30	0.11	2.73	0.39	14.52	0.13
Zinc[a]	38.61	63.49	59.09	44.00	44.29	61.69	41.70	61.47	46.00	63.89	36.15	63.80
Ruthenium	1.26	b.d.	6.27	b.d.	8.52	b.d.	7.37	b.d.	7.02	0.01	0.86	b.d.
Rhodium	0.41	b.d.	5.49	0.02	7.88	0.01	6.72	b.d.	6.27	0.01	0.40	b.d.
Silver	0.73	0.45	1.28	0.29	1.58	1.03	0.62	0.33	0.98	0.41	1.23	0.85
Cobalt[a]	0.86	0.46	5.04	0.05	7.08	0.16	6.37	b.d.	5.96	0.04	0.72	0.05
Molybdenum[a]	0.02	0.09	1.05	1.40	2.33	0.50	0.75	0.09	3.46	0.08	0.02	0.22
Tellurium	b.d.	b.d.	1.44	b.d.	1.83	b.d.	1.00	b.d.	0.90	b.d.	0.00	0.00
Trace (ng·g^{-1})												
Germanium	b.d.	35	b.d.	28	b.d.	92	b.d.	123	b.d.	142	b.d.	44
Yttrium	62	29	113	11	182	11	112	b.d.	136	195	157	14
Cadmium	483	22	466	29	345	63	301	35	292	12	853	33
Dysprosium	29	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	47	b.d.
Vanadium[a]	b.d.	b.d.	718	295	560	591	979	251	1011	20	b.d.	355
Lead	369	24	664	153	518	51	876	57	380	28	186	58
Tin[a]	b.d.	60	243	391	287	212	b.d.	69	b.d.	195	186	127
Neodymium	b.d.	255	b.d.	b.d.	b.d.	12	b.d.	b.d.	b.d.	7	104	b.d.
Europium	b.d.	b.d.	109	b.d.	169	b.d.	113	b.d.	93	b.d.	b.d.	b.d.
Lanthanum	b.d.	318	b.d.	14	6	20	6	6	b.d.	12	b.d.	14

[a]Essential element.

iron, boron, titanium, selenium, iodine, manganese, and barium during the growth and development of the spot prawn (Table 1). Larvae contained less quantifiable elements than did the hepatopancreas or abdomen tissue of the parent spot prawns. Only 0.6 μg strontium·g^{-1} and 0.02 μg bromine·g^{-1} were present in the larvae, but these were normally major elements in the spot prawn. All essential elements were abundant in larvae (Table 1). Germanium in the larvae may have been accumulated from the seawater in the laboratory, as it was present at 25 ng·g^{-1} in salts obtained from evaporation of the seawater. The high levels of lead, caesium, bismuth, antimony, and zirconium in the newly hatched larvae must have been derived from the egg, or parent, because their concentration in laboratory seawater salts was less than 1 ng·g^{-1}.

Substantial differences occurred in the elemental composition of the body parts of the post-hatch female spot prawns (Table 1). Calcium and magnesium were the dominant elements in all body segments. A higher number of quantifiable elements were contained in the chitinous carapace than in the soft tissue, and in general the carapace had higher concentrations of metals (Table 1). Both bromine and iodine were accumulated almost exclusively in the exoskeletal segments. The essential elements were contained in lower or comparable concentrations in the hepatopancreas than in other parts of the body except for copper, arsenic, and molybdenum. The hepatopancreas also accumulated higher levels of heavy metals such as cadmium, mercury, samarium, niobium, and tungsten than other segments of the body (Table 1).

Considerable variation occurred in concentrations of elements in the abdominal carapaces of different size classes of the spot prawn, irrespective of collection site (Table 2). The variability in carapace elements of different size classes was considered a function of molting frequency, with accumulation of elements reflecting the formation and age of the carapace. The precise age of the carapaces of immature and mature males was difficult to determine. However, females carrying eggs on their pleopods from the previous autumn could not have molted for at least 6 mo. Therefore, the female carapace provided an elemental composition reflecting longer term characterization of populations at different coastal sites.

A comparison of elemental profiles of female carapaces of spot prawns collected from five different locations showed that specimens from Snake Bay, in Sechelt Inlet, were unique. Snake Bay spot prawn carapaces had higher levels of calcium, sodium, strontium (Fig. 2), boron, barium (Fig. 3), dysprosium, neodymium, iridium, and cadmium (Fig. 4) and lower levels of titanium, scandium, ruthenium, cobalt (Fig. 3), tellurium, europium, vanadium, and molybdenum (Fig. 4) relative to concentrations of these elements in specimens from the other locations. Similarities in elemental composition of female carapaces from the remaining collection sites indicated their subdivision into two groups: (1) Porteau Cove with Cotton Point which are both in Howe Sound and (2) Dakota Creek which is in Howe Sound with Thornhill Point which is in Salmon Inlet (Fig. 2–4).

By contrast, lower and more constant levels of elements were contained in the abdominal tissue of different size classes of spot prawn within and between the different populations investigated (Table 2). The coefficients of variation of levels of calcium, sodium, iron, titanium, chromium, selenium, iodine, scandium, manganese, and copper in the tissue of spot prawns from both locations were about 50% or less than corresponding values for the carapaces.

The fatty acid profile of the hepatopancreas differed from similar profiles exhibited by the head, abdomen carapace, and abdomen tissue of female spot prawns (Table 3). The principal fatty acids in the body parts were 16:0, 18:1*n*9, 18:1*n*7, 20:4*n*6, 20:5*n*3, and 22:6*n*3, with over 80% of the polyethylenic acids composed of the *n*3 series of PUFA's. The approximately 12% reduction in PUFA's in the hepatopancreas was offset by an increased level of monoethylenic acids, particularly 16:1*n*7, 20:1*n*11, 20:1*n*9, 20:1*n*7, and 22:1*n*11.

Fatty acid profiles of abdominal tissue of immature males, mature males, and female spot prawns collected from different

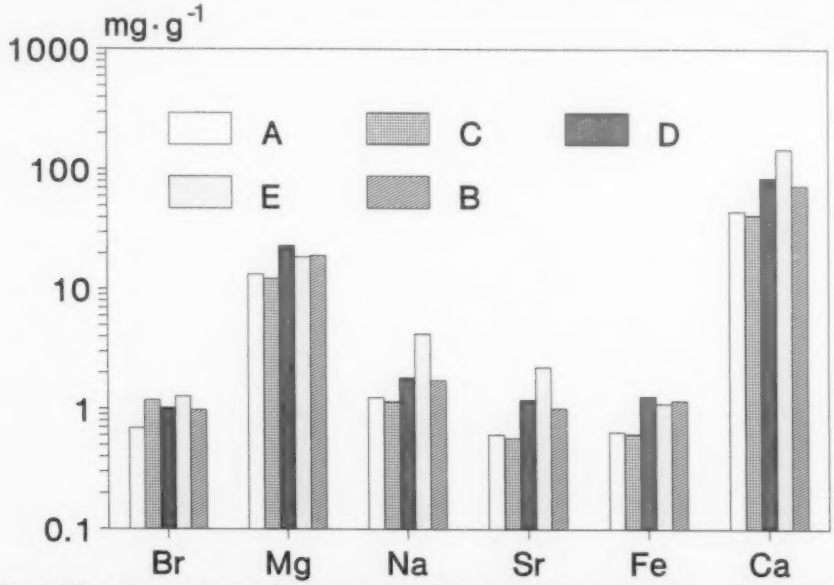

FIG. 2. Major elements in the abdomen carapaces of female spot prawns collected from Porteau Cove (A), Dakota Creek (B), Cotton Point (C), Thornhill Point (D), and Snake Bay (E).

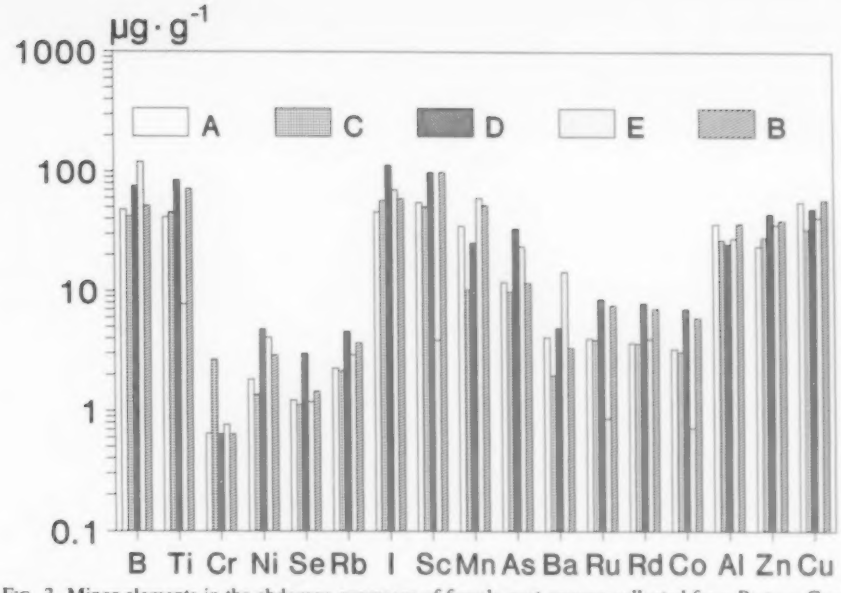

FIG. 3. Minor elements in the abdomen carapaces of female spot prawns collected from Porteau Cove (A), Dakota Creek (B), Cotton Point (C), Thornhill Point (D), and Snake Bay (E).

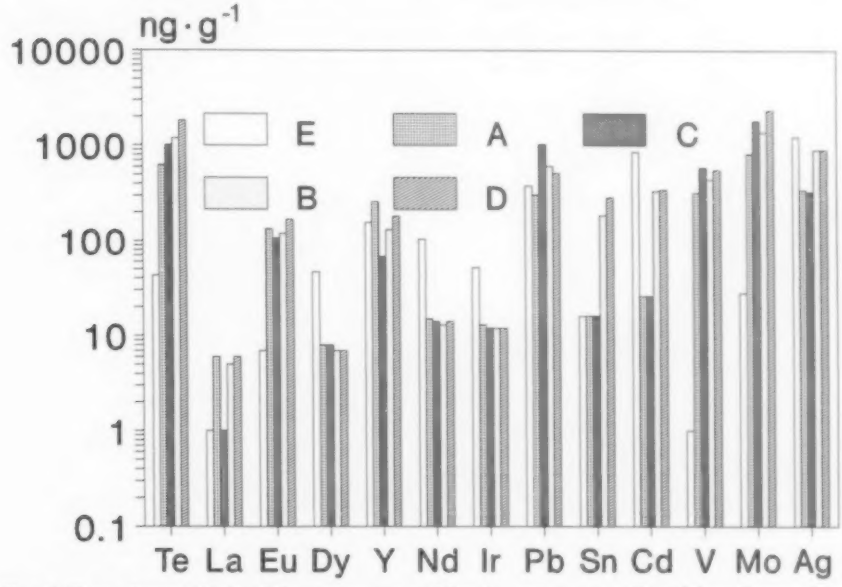

FIG. 4. Trace elements in the abdomen carapaces of female spot prawns collected from Porteau Cove (A), Dakota Creek (B), Cotton Point (C), Thornhill Point (D), and Snake Bay (E).

locations were remarkably similar (Table 4). No significant difference ($P > 0.05$) was seen in the mean values of fatty acids from the four collection sites. Nevertheless, multiple comparison of mean values for fatty acids in immature, mature, and females, independent of sites, demonstrated significant differences in the percentage of iso-17:0 and 18:1*n*9 between all size classes and 16:3*n*4, 16:4*n*3, 18:1*n*11, 18:1*n*7, 18:2*n*6, 18:3*n*3, and 20:5*n*3 between immature males and females (Table 4). The increase in content of iso-17:0 and 18:1*n*9 provided the significant increase in monoethylenic acids with maturity. The significant decrease with maturity in HUFA's of the *n*3 series was compensated for by the increase in the *n*6 series.

Discussion

Detailed multielement analysis of decapod crustaceans has been limited to mesopelagic species (White and Rainbow 1987; Ridout et al. 1985, 1989). Except for chromium the levels of elements in the larvae of the spot prawn were within or below the range of 10 elements cited for nine mesopelagic species (Table 5). By contrast, all these elements except for arsenic, cadmium, and vanadium were in higher concentration in the

TABLE 3. Percentage fatty acids in the total lipids of abdomen tissue, abdomen carapace, head, and hepatopancreas of post-hatch female *P. platyceros* from Copper Bluff.

Fatty acids	Abdomen tissue	Abdomen carapace	Head	Hepatopancreas
14:0	0.74	0.54	0.54	1.61
15:0	0.53	0.31	0.33	0.54
iso-16:0	0.24	0.20	0.20	0.20
16:0	15.98	13.30	12.19	11.68
16:1*n*7	4.38	4.02	4.11	8.96
iso-17:0	0.84	0.74	0.70	1.59
Aiso-17:0	0.44	0.39	0.38	1.13
17:0	1.06	0.98	0.81	0.71
16:3*n*4	0.81	0.81	0.70	0.93
16:4*n*3	2.26	2.05	2.15	0.20
Unknown	0.86	0.83	0.90	0.20
18:0	3.80	3.92	4.06	3.80
18:1*n*11	0.20	0.21	0.20	0.21
18:1*n*9	14.62	14.92	14.62	15.89
18:1*n*7	6.53	7.03	6.60	6.70
18:1*n*5	0.46	0.39	0.42	0.77
18:2*n*6	1.28	1.50	1.47	2.33
18:2*n*4	0.24	0.20	0.21	0.36
18:3*n*3	0.24	0.27	0.26	0.44
20:1?	0.38	0.41	0.43	0.42
20:1*n*11	0.42	0.34	0.43	2.09
20:1*n*9	0.59	0.62	0.70	3.97
20:1*n*7	0.58	0.62	0.70	3.86
20:2*n*6	0.20	0.22	0.21	0.48
20:4*n*6	3.80	4.70	5.13	2.43
20:4*n*3	0.21	0.20	0.20	0.23
20:5*n*3	18.35	19.21	20.19	8.09
22:1*n*11	0.34	0.28	0.29	2.16
21:5*n*3	0.20	0.21	0.24	0.22
22:4*n*6	0.31	0.34	0.48	0.86
22:5*n*6	0.34	0.41	0.43	0.57
22:5*n*3	0.97	0.93	1.20	1.33
22:6*n*3	11.01	12.61	11.99	8.22
Saturated	23.63	20.38	19.21	21.26
Monoethylenic	28.50	28.84	28.50	45.03
Polyethylenic	40.22	43.66	44.86	26.69
Σ4–6,*n*3/poly.	82.05	80.65	80.18	68.53
Σ4–6,*n*6/poly.	11.06	12.48	13.46	14.46

whole post-hatch female spot prawns than in the oceanic species. Copper, zinc, and cadmium levels in the post-hatch female spot prawns were also within the range cited for other coastal crustaceans (Table 5). The pelagic and benthic stages of the spot prawn therefore contained levels of some minor elements which were consistent with those recorded in the literature for other crustaceans.

The high copper level in the hepatopancreas of the post-hatch female spot prawns was presumed to result from starvation. Djangmah (1970) demonstrated that during starvation of *Crangon vulgaris* the breakdown of haemocyanin resulted in copper accumulation in the hepatopancreas. All essential elements in the newly hatched larvae were parentally derived via the eggs, as these elements were present at less than 1 $ng \cdot g^{-1}$ in dry salts from the ambient seawater. Levels of iron, manganese, copper, and zinc in the larvae were lower respectively than the 27.4, 3.9, 83.7, and 70.8 $\mu g \cdot g^{-1}$ which are considered nutritional requirement levels for crustaceans (White and Rainbow 1987). The essential nature of caesium, bismuth, antimony, and zirconium in spot prawns may invite future queries, as observed levels in the newly hatched larvae could only have originated from the eggs and broodparent.

Hardening of crustacean carapace results from a process in which inorganic elements, mainly calcium, are embedded in the organic matrix (Stevenson 1985). Size, age, and reproductive condition of some species of bivalves and mesopelagic crustaceans have been correlated with accumulation of many elements (Boyden 1977; Ridout et al. 1989). In the spot prawn, accumulation of elements was not correlated with size, age, or sex, but varied with the part of the body analysed. The protein–chitinous exoskeleton provided the greatest number of quantifiable elements. However, preferential absorption and desorption or equilibration of elements with carapace age must occur because many elements were at lower levels in the carapaces of females than in the more frequently molted carapaces of the immature and mature males from the same collection site. The female carapace was therefore a more effective long-term monitor of inorganic elements in populations at any given site.

Bryan (1967) from a study of zinc regulation in the crayfish *Austropotamobius pallipes pallipes* concluded that zinc in the body was obtained from the diet and that zinc in the carapace was absorbed directly from ambient water. This mode of accumulation could explain the more constant levels of elements in abdomen tissue as opposed to the more variable nature of elements in carapace of different size classes of the spot prawn.

The substantially different profile of carapace elements in spot prawns from Snake Bay relative to the similar profiles obtained from Porteau Cove and Cotton Point animals suggested the possibility of separate stocks in the geographically separate inlets (Fig. 1). Spot prawns from Dakota Creek and Thornhill Point exhibited similar elemental profiles, which were quite distinct from all other profiles, yet it was unlikely that these spot prawns from widely separate geographic locations could be from the same stock. As carapace elements are derived mainly from absorption, this apparent anomaly may be explained by considering the source of elements in the ambient seawater at the collection sites. In areas with high precipitation the physicochemical and biological processes in coastal waters are influenced continually by the organic and inorganic components carried by the freshwater runoff (Drinkwater 1986). Although physically separated, the seawater at Dakota Creek and Thornhill Point would be influenced by the freshwater runoff from the same mountain watershed on the west side of Howe Sound. The freshwater runoff from the mountain watershed on the east side of Howe Sound would influence the seawater composition at Porteau Cove and Cotton Point and would be distinct from the seawater composition at Snake Bay at the top of Sechelt Inlet (Fig. 1). Carapace elements, even of egg-carrying females, are not indicative of spot prawn stocks, but represent the quality of water at the collection site.

The fatty acid profile of *P. platyceros* was similar to that of *P. borealis*, except for higher levels of the essential fatty acids eicosapentaenoic acid (20:5*n*3) and docosahexaenoic (22: 6*n*3), acid in the former (Ackman and Eaton 1967). The constant high level of HUFA's in body parts from female spot prawns starved for 28 d and site-collected prawns indicated initial high assimilation of HUFA's from the diet and a stability to short-term inanition which made fatty acids suitable as markers. Hepatopancreas lipid is a major source of energy in starved spot prawns (Whyte et al. 1986) and accounted for the reduced level of HUFA's, particularly 20:5*n*3, in the starved spot prawns. Minor differences in fatty acid profiles of different size classes of spot

TABLE 4. Percentage fatty acids in the total lipids of abdomen tissue from immature male, mature male, and female *P. platyceros* collected from four different locations. Newman–Keuls multiple comparison of mean values from immature males, mature males, and females is represented by superscripted letter(s) in the Dakota Creek columns. The same letter(s) indicated no significant difference in the means from one another ($P > 0.05$); those with different letters were significantly different ($P < 0.05$).

	Immature males				Mature males				Females			
Fatty acids	Dakota Creek	Cotton Point	Thornhill Point	Snake Bay	Dakota Creek	Cotton Point	Thornhill Point	Snake Bay	Dakota Creek	Cotton Point	Thornhill Point	Snake Bay
14:0	0.94[a]	0.87	1.29	1.23	0.93[a]	0.90	0.97	1.40	0.72[a]	0.74	0.89	0.97
15:0	0.63[a]	0.54	0.49	0.56	0.57[a]	0.51	0.59	0.54	0.43[a]	0.42	0.58	0.49
iso-16:0	0.86[a]	1.14	1.18	1.12	1.00[a]	1.09	1.19	1.12	1.24[a]	1.32	1.45	1.19
16:0	15.61[a]	16.33	17.54	17.60	16.01[a]	16.17	17.28	17.24	14.88[a]	15.94	15.61	16.58
16:1*n*7	4.86[a]	3.57	4.22	4.39	4.84[a]	4.34	4.66	4.67	5.68[a]	4.12	5.22	5.24
iso-17:0	0.40[a]	0.42	0.42	0.42	0.55[b]	0.53	0.63	0.56	0.71[c]	0.59	0.71	0.70
Aiso-17:0	0.37[a]	0.25	0.30	0.31	0.47[a]	0.34	0.37	0.32	0.59[a]	0.36	0.30	0.43
17:0	1.14[a]	0.95	0.89	0.94	1.09[a]	0.92	0.70	0.84	1.03[a]	0.82	0.90	0.78
16:3*n*4	0.65[a]	0.52	0.52	0.57	0.77[b]	0.65	0.73	0.64	0.99[b]	0.64	0.78	0.82
16:4*n*3	2.31[a]	2.52	2.66	2.37	2.06[b]	1.98	1.98	1.78	2.09[b]	2.00	1.88	1.71
Unknown	0.78[a]	0.59	0.55	0.55	0.78[a]	0.67	0.72	0.58	0.98[b]	0.83	1.03	0.84
18:0	3.40[a]	3.66	3.91	3.72	3.38[a]	3.42	3.58	3.24	3.35[a]	3.63	3.61	3.24
18:1*n*11	0.26[a]	0.30	0.22	0.34	0.26[ab]	0.26	0.20	0.21	0.22[b]	0.20	0.20	0.20
18:1*n*9	9.56[a]	10.07	11.00	10.71	11.17[b]	11.34	14.08	11.83	14.55[c]	15.05	14.64	16.34
18:1*n*7	8.32[a]	6.13	5.97	6.10	7.44[ab]	6.45	5.76	5.75	7.13[b]	6.65	5.76	5.93
18:1*n*5	1.02[a]	0.37	0.32	0.38	0.61[a]	0.51	0.38	0.38	0.45[a]	0.36	0.38	0.41
18:2*n*6	1.70[a]	1.46	1.16	1.68	1.29[ab]	1.38	1.10	1.26	1.33[b]	0.95	0.93	0.97
18:2*n*4	0.33[a]	0.22	0.27	0.23	0.32[a]	0.39	0.26	0.22	0.28[a]	0.28	0.23	0.22
18:3*n*3	0.59[a]	0.67	0.37	0.62	0.42[ab]	0.48	0 31	0.52	0.30[b]	0.25	0.34	0.29
20:1?	0.41[a]	0.27	0.28	0.29	0.52[a]	0.38	0.48	0.23	0.62[a]	0.20	0.33	0.29
20:1*n*11	0.51[a]	0.45	0.41	0.56	0.60[a]	0.68	0.49	0.61	0.68[a]	0.62	0.36	0.61
20:1*n*9	0.28[a]	0.42	0.84	0.87	0.40[a]	0.42	0.64	1.08	0.23[a]	0.39	0.83	0.72
20:1*n*7	0.50[a]	0.44	0.41	0.42	0.59[a]	0.45	0.58	0.55	0.55[a]	0.48	0.63	0.52
20:2*n*6	0.30[a]	0.41	0.35	0.44	0.24[a]	0.26	0.24	0.31	0.25[a]	0.21	0.20	0.23
20:4*n*6	3.04[a]	4.03	3.52	3.79	3.15[a]	3.78	3.82	3.67	4.19[a]	5.27	3.84	4.08
20:4*n*3	0.22[a]	0.23	0.20	0.24	0.20[a]	0.20	0.20	0.20	0.20[a]	0.20	0.20	0.20
20:5*n*3	21.37[a]	21.96	21.98	20.61	21.28[b]	20.40	18.27	19.46	19.73[b]	18.43	19.72	18.42
22:1*n*11	0.22[a]	0.20	0.38	0.52	0.28[a]	0.30	0.30	0.57	0.21[a]	0.25	0.32	0.40
21:5*n*3	0.21[a]	0.30	0.20	0.24	0.22[a]	0.27	0.20	0.20	0.21[a]	0.20	0.20	0.20
22:4*n*6	0.26[a]	0.46	0.39	0.53	0.28[a]	0.38	0.45	0.48	0.49[a]	0.34	0.31	0.52
22:5*n*6	0.24[a]	0.27	0.24	0.28	0.28[a]	0.34	0.39	0.31	0.35[a]	0.89	0.37	0.30
22:5*n*3	0.96[a]	1.38	1.48	1.38	1.01[a]	1.12	1.21	1.22	1.06[a]	1.03	1.19	1.26
22:6*n*3	12.18[a]	14.58	12.14	11.83	12.38[a]	13.98	12.72	13.29	9.57[a]	12.18	11.66	10.60
Saturated	23.35[a]	24.16	26.02	25.90	24.00[a]	23.88	25.31	25.26	22.95[a]	23.82	24.05	24.38
Monoethylenic	25.94[a]	22.22	24.05	24.58	26.71[b]	25.13	27.57	25.88	30.32[c]	28.32	28.67	30.66
Polyethylenic	44.36[a]	49.01	45.48	44.81	43.90[ab]	45.61	41.88	43.56	41,04[b]	42.87	41.85	39.82
Σ4–6,*n*3/poly.	83.97[a]	83.60	85.00	81.83	84.62[a]	83.21	82.57	82.99	80.07[a]	79.40	83.27	81.34
Σ4–6,*n*6/poly.	7.98[a]	9.71	9.12	10.26	8.45[a]	9.87	11.13	10.24	12.26[b]	15.16	10.80	12.31

prawns, reflecting age and frequency of molt cycle, and the lack of any significant difference in tissue fatty acids from different locations suggested similar diets for spot prawns at all locations. The gradual changes in fatty acid profiles of the shrimps *Penaeus setiferus*, *P. aztecus*, and *P. duorarum* during a 1-yr study period was concluded to result from variation in the food chain rather than from endogenous adjustment to fatty acids from changes in water temperature (Bottino et al. 1980). Feeding trails in the same study confirmed that diet exerted a profound and lasting influence on the fatty acid composition of shrimp body lipids. Fatty acids, particularly the C-20 and C-22 polyunsaturated isomers, in the marine environment originate largely from the primary producers and are altered only slightly by the subsequent consumers (Mayzaud et al. 1989). The similarity in fatty acid profiles of the spot prawn indicated similarity in diets in all collection sites. This strengthened the earlier suggestion that abdomen tissue elements, which were also similar in spot prawns from different locations, originated mainly from diet. However, no relationship between carapace elements and diet was evident.

The data in this study suggested an active accumulation of strontium, bromine, iron, boron, titanium, selenium, iodine, manganese, and barium during the growth and development of the spot prawn. The carapace of female spot prawns was the

TABLE 5. Concentrations of minor elements in dry whole crustaceans. Superscript numbers correspond to the lower or upper limit values provided by the species: [1]*Eucopia sculpticauda*, [2]*Acanthephyra purpurea*, [3]*Thysanopoda microphthalma*, [4]*Systellaspis debilis*, [5]*Gennadas valens*, [6]*Bentheogennema intermedia*, [7]*Eucopia unguiculata*, [8]*Meganyctiphanes norvegica*, [9]*Nematoscelis megalops*, [10]*Crangon vulgaris*, [11]*Palaemon elegans*, [12]*Palaemon serratus*.

Elements ($\mu g \cdot g^{-1}$)	Limits for mesopelagic spp.[a]	Limits for coastal spp.[b]	*P. platyceros*[c]	
			Larvae	Females
Iron	17.3[1]–92.7[2]	—	3.00	351.8
Chromium	0.17[3]–0.79[4]	—	1.31	2.82
Nickel	0.57[3]–2.28[5]	—	0.51	2.58
Manganese	1.20[1]–4.00[6]	—	0.80	10.90
Copper	20.7[7]–71.9[8]	64.7[10]–129.2[11]	27.40	95.10
Arsenic	17.4[5]–59.3[8]	—	39.30	33.44
Zinc	23.9[1]–96.5[8]	77.8[12]–118.9[10]	56.09	102.51
Cadmium	0.20[9]–13.3[4]	0.31[11]–12.20[10]	0.13	1.24
Cobalt	0.04[1]–0.22[4]	—	0.22	0.31
Vanadium	0.17[8]–1.84[4]	—	0.60	0.68

[a]Values taken from Ridout et al. (1989).
[b]Values taken from White and Rainbow (1982).
[c]Values from Table 1 and calculated for whole female spot prawns.

best monitor of long-term elemental environments at any location. The similarity in fatty acids in body parts of spot prawns from different locations suggested uniformity of diet which was supported by the similarity in elemental composition of the abdomen tissue from different locations. Considered collectively the composition of elements absorbed into the carapace of female spot prawns from separate geographic locations was more likely to arise from the elemental composition of the ambient seawater, rather than from the diet, or from any variability associated with element metabolism in different stocks of the spot prawn.

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Modèle énergétique uniboite de la croissance des huîtres (*Crassostrea gigas*) dans le bassin de Marennes-Oléron

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Bacher, C., M. Héral, J. M. Deslous-Paoli et D. Razet. 1991. Modèle énergétique uniboite de la croissance des huîtres (*Crassostrea gigas*) dans le bassin de Marennes-Oléron. Can. J. Fish. Aquat. Sci. 48: 391–404.

Un modèle de croissance des huîtres (*Crassostrea gigas*) dans le bassin de Marennes-Oléron est élaboré afin d'étudier la notion de capacité trophique. Les facteurs environnementaux pris en compte sont le seston total et la somme des équivalents énergétiques des protides, lipides et glucides de la colonne d'eau. Leur évolution temporelle sur 2 ans est reconstituée avec un pas de temps journalier à l'aide d'un modèle régressif fonction du courant de marée, de la hauteur d'eau et de la force du vent. A partir de ces séries, un modèle de bilan énergétique, comprenant un terme d'assimilation et un terme de respiration, est construit pour simuler la croissance d'une huître pendant 2 ans. Connaissant le nombre d'individus dans le bassin, le débit entrant et le volume moyens des masses d'eau dans le bassin, ce modèle permet d'estimer à 7 % le pourcentage d'énergie utilisée par la population. En faisant varier de façon théorique l'effectif de la population, on montre que la croissance individuelle est sensible au stock d'huîtres. Ce calcul introduit la notion de capacité trophique et montre l'intérêt d'une approche plus élaborée de couplage d'un modèle de transport de la nourriture et du modèle de croissance étudié.

The growth of oysters (*Crassostrea gigas*) is modeled in order to study the trophic capacity of the Bay of Marennes-Oléron. Total seston and amount of protids, lipids, and carbohydrates converted to energy are the environmental factors taken into account. Time series of these variables are derived during 2 yr with a daily time step from a linear regression including the tidal velocity, the height of the water column, and the wind velocity. A model of the energetic budget based on assimilation and respiration terms and depending on the previous time series is run to simulate the individual growth of an oyster. The knowledge of the number of oysters, the flow of water entering the bay, and the volume of the bay allows us then to estimate that 7% of the available energy is used by the population. The number of individuals is given theoretical values to show that the impact of the amount of oysters on the growth of an individual is nonnegligible. The trophic capacity of the bay is then defined and it is pointed out that more information may be expected from a model coupling the transport of particulate matter and the growth of oysters.

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Les relations trophiques des bivalves ont le plus souvent été modélisées de façon empirique. Ainsi Bodoy et Plante-Cuny (1984), Héral *et al.* (1984) établissent des régressions linéaires du poids individuel en fonction des paramètres du milieu afin de déterminer les paramètres hydrobiologiques ou sédimentologiques les plus importants. Goulletquer et Bacher (1989) généralisent cette démarche par l'introduction de régressions non linéaires et comparent les performances de croissance en différents sites. Ces modèles empiriques peuvent être prédictifs et généralement applicables à toute étude de relations trophiques dans le même site ou un site de caractéristiques hydrobiologiques voisines. Ils manquent cependant de réalisme (Levins 1966) et ne permettent pas de mesurer la quantité de nourriture retirée du milieu par les filtreurs. Il est cependant notable que la nourriture devient limitante pour un stock élevé (Héral *et al* 1986*a*, 1988) et une augmentation de stock induit un ralentissement de croissance.

Ainsi dans le bassin de Marennes-Oléron, important centre de production d'huîtres (*Crassostrea gigas*), la relation entre le stock d'huîtres et les performances de croissance permet d'expliciter la notion de capacité trophique du bassin, définie comme la capacité d'élevage d'un stock initial d'individus devant être amenés à une taille commerciale déterminée. Les modèles de bilan énergétique permettent d'établir un bilan des besoins et dépenses énergétiques globaux (Rodhouse 1979). Cela implique l'identification de la nourriture potentielle et l'écriture des lois de consommation, d'assimilation et de respiration en fonction des paramètres environnementaux. Ce type de modèle a été appliqué à *Mytilus edulis* par Bayne *et al.* (1976), Verhagen (1982) et Harris *et al.* (1984). On trouve également leur utilisation en aquaculture pour certaines espèces de poissons (Majkowski et Waiwood 1981; Kitchell et Breck 1980).

Cette étude a pour but le calage d'un modèle simple de croissance et d'assimilation de *Crassostrea gigas* à l'aide de données acquises principalement *in situ* en 1979 et 1980. Le problème de la variabilité hydrobiologique des variables explicatives du bilan énergétique en milieu estuarien est pris en compte. Le modèle est appliqué de façon théorique au bassin de Marennes-Oléron considéré comme un "raceway" de débit connu afin de calculer l'impact potentiel de l'évolution du stock sur la croissance (schéma 1).

Matériels et méthodes

Plan d'échantillonnage

Les données hydrobiologiques ont été acquises en un point central du bassin (banc Dagnas, fig. 1) par Héral *et al.* (1983a)

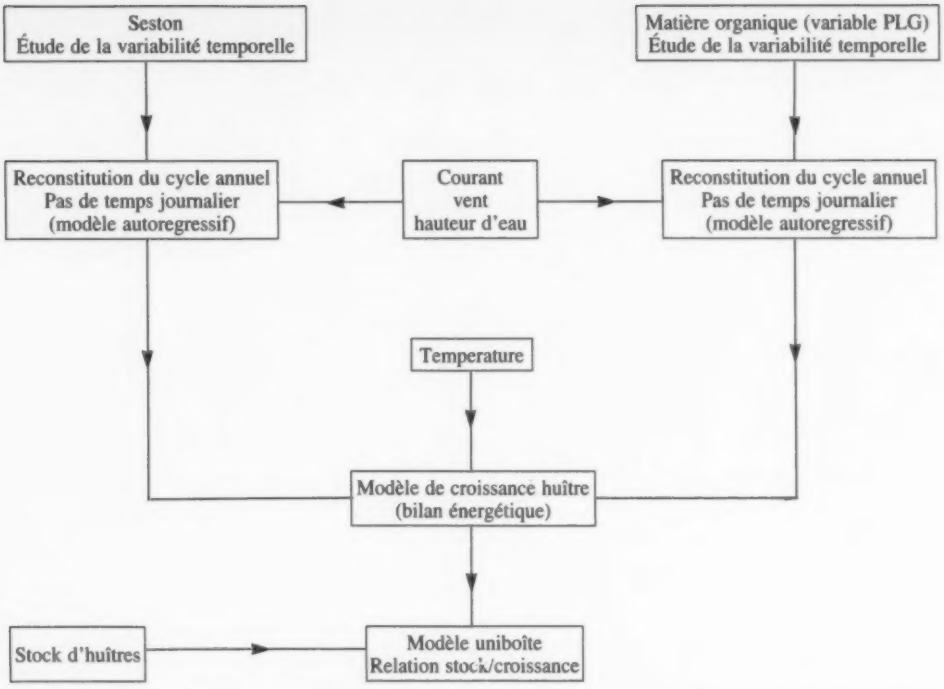

SCHÉMA 1. Organigramme des différents calculs réalisés dans cette étude afin de modéliser la relation entre le stock et les performances de croissance des huîtres *Crassostrea gigas* dans le bassin de Marennes-Oléron.

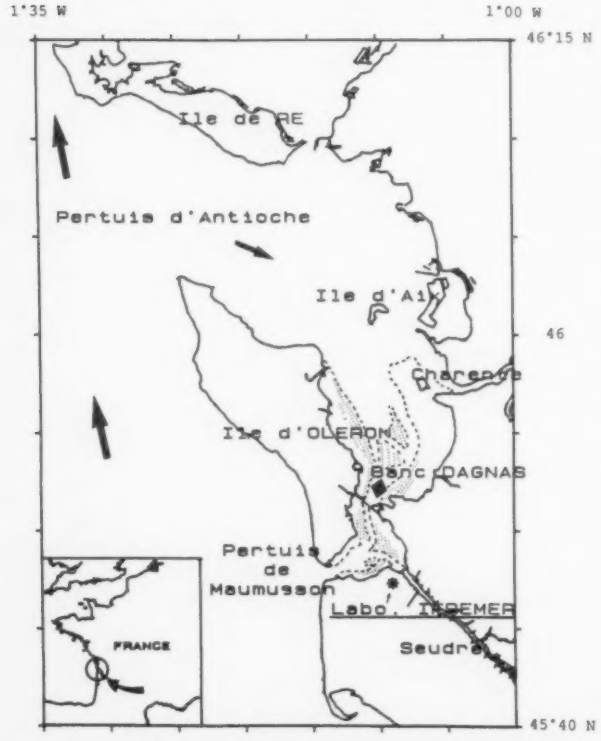

FIG. 1. Carte du bassin de Marennes-Oléron et emplacement du site étudié.

et Deslous-Paoli et Héral (1984). Le plan d'échantillonnage a consisté à suivre une population d'huîtres (200 individus/m^2) et les paramètres de la colonne d'eau pendant deux ans, en 1979 et 1980. Des prélèvements mensuels sur 10 individus ont servi à mesurer le poids sec sans cendre, le poids de coquille, les constituants biochimiques et la valeur énergétique de la chair. Les paramètres hydrobiologiques seston organique, seston minéral, protides, lipides, glucides, température, chlorophylle *a*, phéopigments ont été mesurés à deux hauteurs de la colonne d'eau selon un rythme bimensuel, pendant une marée de vive-eau et une marée de morte-eau. A chacune de ces marées six prélèvements espacés de 2 h furent effectués de façon à estimer la variabilité horaire.

La nourriture potentielle est définie comme la somme des équivalents énergétiques des protides, lipides, glucides de la colonne d'eau (Héral 1985). Par la suite cette variable sera appelée PLG.

Variabilité du milieu

Le bassin de Marennes-Oléron est de type macrotidal. D'après Héral *et al.* (1987), quatre sources de variabilité expliquent les cycles des traceurs particulaires : gradient vertical, cycle horaire de la marée (période 12 : 30), cycle lunaire (période 15 jours) et tendance saisonnière (période 1 an). Une analyse de variance (ANOVA) sur les différentes composantes du seston et de la variable PLG en 1979 et 1980 a permis de hiérarchiser les effets des différentes perturbations d'après les niveaux de signification des trois facteurs date, type de marée (morte-eau ou vive-eau) et hauteur du prélèvement. Le modèle s'écrit

$$Y_{ijk} = + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + \epsilon_{ijk}$$

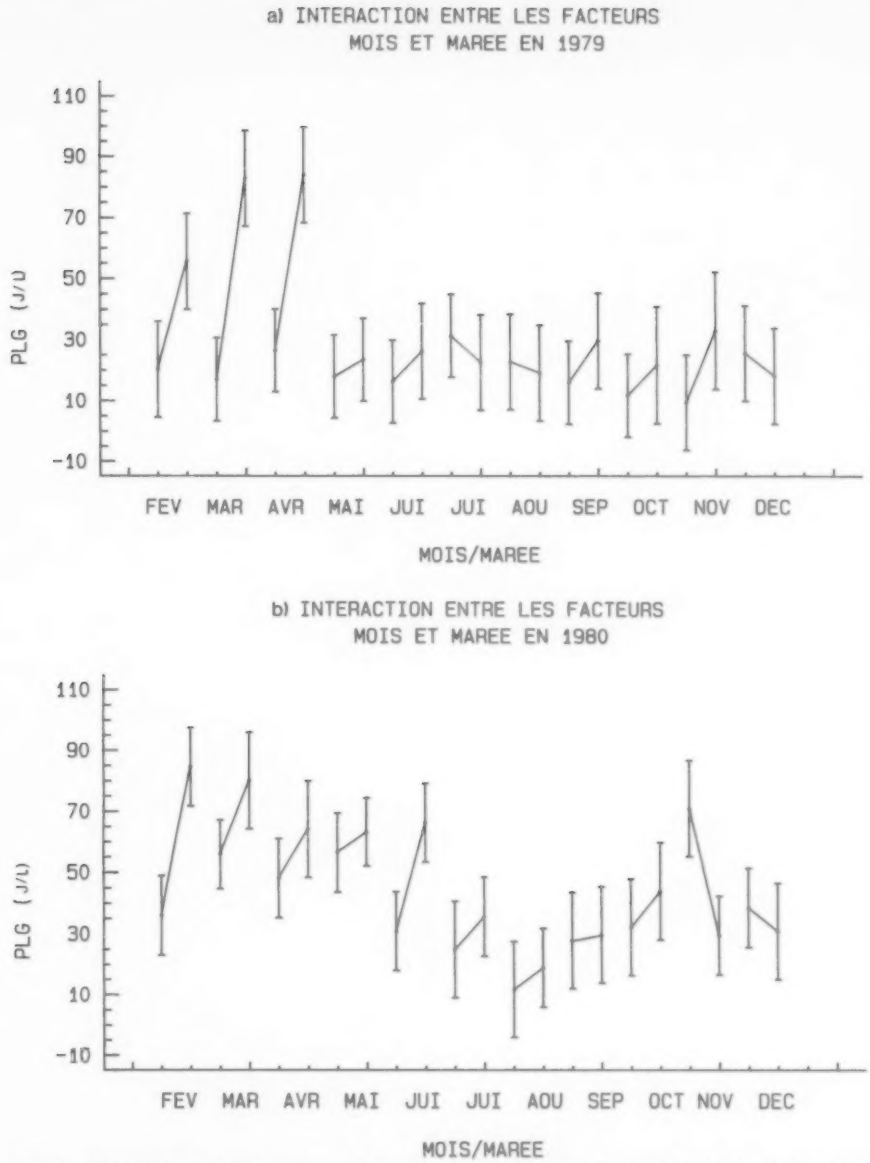

FIG. 2. ANOVA des variables seston et PLG en fonction des facteurs date (mois de février à décembre) et coefficient de marée (mortes-eaux, vives-eaux) en 1979 et 1980. Le graphe met en évidence l'interaction entre les deux facteurs, l'augmentation des concentrations particulaires par marées de vives-eaux et l'évolution saisonnière des variables. Pour chaque mois, la première valeur se rapporte à la marée de mortes-eaux, la deuxième à celle de vives-eaux. a) Variable PLG en 1979; b) variable PLG en 1980; c) variable seston en 1979; d) variable seston en 1980.

où A_i, B_j, et C_k désignent les trois facteurs hauteur du prélèvement (A_1 = surface, A_2 = fond), type de marée (B_1 = morte-eau, B_2 = vive-eau) et date (mois de janvier à décembre), AB_{ij}, AC_{ik} et BC_{jk} les interactions deux à deux et ϵ_{ijk} le résidu.

Le pas de temps des phénomènes biologiques est de l'ordre de la journée. Bien que le temps de réponse des mollusques filtreurs aux fluctuations rapides du milieu n'ait pas été étudié, les données de temps de transit intestinal, entre 2 et 30 h chez *Mytilus edulis* (Bayne *et al.* 1987) ou chez *Crassostrea gigas* (Boucaud-Camou *et al.* 1985; Le Gall, comm. pers.) sont des indicateurs de l'effet intégrateur de l'individu. En fonction de ce pas de temps et de la variabilité du milieu, il est nécessaire de reconstituer le cycle des variables hydrologiques, seston et PLG. Un modèle composé d'un facteur autorégressif et de termes non linéaires comprenant les différentes composantes des vitesses du vent et du courant selon les orientations est–ouest et nord–sud, responsables du transport particulaire et de la remise en suspension, a été utilisé. Le vent marque une tendance saisonnière, mais son évolution journalière est de type stochastique (Bacher 1989). Les mesures de sa direction et de sa vitesse proviennent des enregistrements, toutes les 3 h et à deux stations, fournis par la Météorologie Nationale. Par souci

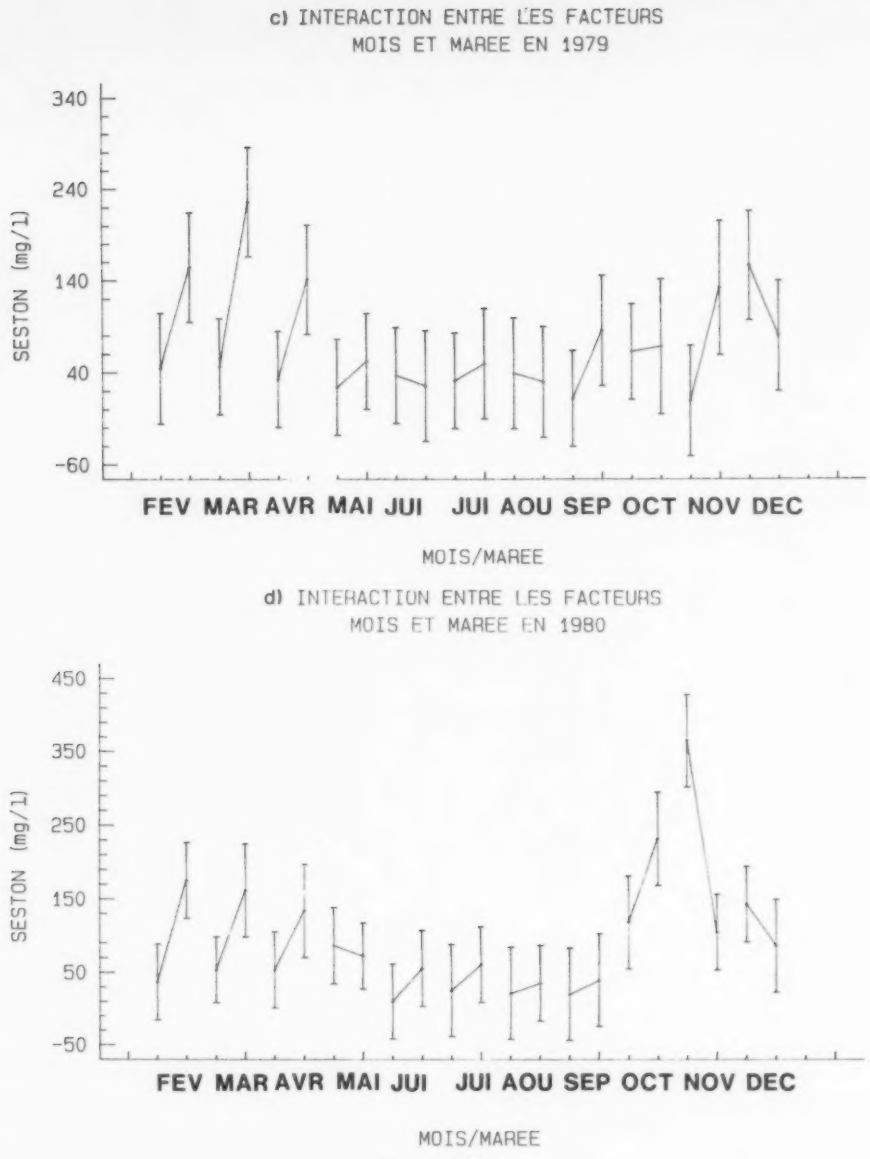

FIG. 2. (*Fin*)

de simplification, ces données ont été moyennées chaque jour. Enfin les courants ont été mesurés aux heures de prélèvement, mais un courant standard (Laboratoire Central Hydraulique de France (LCHF) 1973) a été utilisé dans le modèle, bien qu'il soit sensible au vent et peu variable en fonction du coefficient de marée.

Une fois le modèle calé, une simulation avec un pas de temps de 2 h a été lancée sur les deux années d'étude. Un programme du Service Hydrographique et Océanographique de la Marine (SHOM), adapté sur compatible IBM/PC, a été utilisé pour prédire la hauteur d'eau toutes les 2 h en conditions météorologiques normales. Ce même programme a fourni les hauteurs de la colonne d'eau. La moyenne journalière du seston a été déduite des simulations du modèle autorégressif. Le modèle s'écrit

$$Y_t = f(Y_{t-1}, Z_t, U_t, V_t, U_x, U_y, V_x, V_y, U_x \cdot V_x, U_y \cdot V_y, UV)$$

où f est une fonction linéaire de la hauteur Z, de la vitesse du vent U, de la vitesse du courant mesuré V et de leurs composantes selon les axes ouest–est (x) et sud–nord (y). Les termes non linéaires $U_x V_x$ et $U_y V_y$ ont été également introduits.

Modèle de croissance

Le modèle de bilan énergétique s'écrit

$$(1) \quad dW/dt = (A - R) \cdot p \cdot TIM$$

où W = poids sec converti en énergie (Deslous-Paoli 1980), dW/dt = taux de croissance instantanée (joules par heure), A = assimilation et R = respiration, fonctions du seston (variable S), de la somme des équivalents énergétiques des protides,

TABLEAU 1. Pourcentages de variation du seston et de PLG expliqués par les facteurs date et coefficient de marée et leur interaction dans les ANOVA par année et hauteur de prélèvement.

Année	Variable	Hauteur	
		Surface	Fond
1979	PLG	74	73
	Seston	61	57
1980	PLG	83	80
	Seston	82	78

lipides et glucides de la colonne d'eau (variable PLG), de la température T et de W, p = proportion d'énergie consacrée à la chair, $1 - p$ = proportion consacré à la coquille en fonction de la classe d'âge (Deslous-Paoli 1980) et TIM = fraction de la durée journalière d'immersion. La variable TIM est prédite à l'aide d'une loi empirique calée sur les observations recueillies sur le site et fonction du coefficient de marée.

Peu de travaux sont orientés sur la détermination de la date de ponte et l'intensité de celle-ci. Bayne *et al.* (1976) et Verhagen (1982) proposent des lois allométriques ou fonction de la production ("scope for growth"). Il est vraisemblable cependant que la température joue un rôle dans le déclenchement de la ponte et dans la gamétogenèse (notion de degrés-hours, Héral *et al.* 1986b). D'autre part, il apparait que, si la ponte est pratiquement instantanée pour un individu, elle peut s'étaler sur plusieurs jours au niveau de la population. Faute de données, la date de ponte n'est pas prédite mais imposée selon les observations des années 1979–1980. L'intensité de la ponte est représentée comme la perte d'une fraction du soma, fonction du poids sec à la date de ponte, et étalée dans le temps de sorte que l'effort de reproduction total corresponde aux données de la littérature pour les classes d'âge 1 et 2. La croissance individuelle modélisée représente l'évolution d'un individu moyen sans que soit prise en compte la variabilité de la population. Le terme de respiration est tiré de Boukabous (1983) et ne dépend que du poids sec (fonction allométrique d'exposant R_1) et de la température T (loi exponentielle) :

$$R = R_0 \cdot W^{R_1} \cdot \mathrm{EXP}(R_1 \cdot T).$$

Le terme d'assimilation est plus complexe. Le fonctionnement du bivalve filtreur se décompose en trois étapes : filtration, ingestion et assimilation. Pour l'huître, la filtration est une relation allométrique indépendante des conditions du milieu (Deslous-Paoli *et al.* 1987). Le taux d'ingestion a été explicité par Bayne *et al.* (1976) puis Verhagen (1982) pour *Mytilus edulis* et traduit le fait qu'à charge sestonique croissante, une part croissante de particules consommées est évacuée sous forme de pseudofèces (encombrement des palpes labiaux). Cette action inhibitrice de la charge sestonique S (milligrammes par litre) est formulée ici par une fonction inverse :

$$t_i = \frac{1}{S + S_0}.$$

Le taux d'assimilation t_a dépend de la densité d'organique ingérée (rapport organique/minéral), de la température (Dame *et al.* 1980) et de la qualité de la matière organique (Epifanio

TABLEAU 2. Résultats des régressions linéaires pas à pas du seston total et de la variable PLG en fonction des vitesses de vent et de courant, de la hauteur d'eau et d'un terme autorégressif.

Variable	Coefficient de regression	Probabilité
a) Variable Y = seston total; R multiple = 0,774		
$Y(t-1)$	0,522	$<10^{-3}$
Hauteur	−8,400	0,05
Courant	114,024	0,01
Vent	9,852	$<10^{-3}$
b) Variable Y = PLG; R multiple = 0,791		
$Y(t-1)$	0,697	$<10^{-3}$

1979). La relation entre le taux d'assimilation et les variables du milieu est de la forme

$$t_a = \mathrm{PLG} \cdot \frac{T - T_0}{T_1 - T_0} \cdot \mathrm{EXP}\left(\frac{T_1 - T}{T_1 - T_0}\right)$$

où T désigne la température et PLG la concentration de nourriture convertie en énergie (joules par litre). L'expression de l'influence de la température traduit son effet inhibiteur pour $T > T_1$ ou $T < T_0$ (Newell 1979). La fonction d'assimilation s'écrit finalement

$$A = F_1 \cdot W^{F_2} \cdot t_i \cdot t_a.$$

Le temps d'immersion est directement relié au coefficient de marée C à l'aide de la loi empirique, propre au site étudié :

$$\mathrm{TIM} = p + r \cdot (C - q)^2, \quad \text{si } C > 70$$
$$\mathrm{TIM} = 24, \quad \text{si } C < 70$$

où p, q et r sont estimés à partir des mesures *in situ*. La température est interpolée linéairement en fonction du temps entre les dates de prélèvement.

Les paramètres F_1, F_2, T_0, T_1 et S_0 sont estimés par optimisation. L'exposant de l'allométrie F_2 est de l'ordre de 0.75 (Fiala-Médioni et Copello 1985). L'algorithme utilisé est le simplex (Nelder et Mead 1965) adapté par Schnute (1982), basé sur la minimisation de la somme des carrés des écarts entre observations et prédictions, grâce à une méthode itérative. A partir du lot de paramètres ainsi trouvé, l'équation 1 permet de simuler la croissance sur 2 ans. La régression entre observations et valeurs simulées, en tenant compte du nombre de degrés de liberté nécessaires à l'estimation des paramètres, mesure l'adéquation du modèle.

Modèle monoboite

Le modèle précédent sert à illustrer, de façon théorique, la notion de capacité trophique. En supposant les concentrations des divers types particulaires homogènes dans le bassin, et connaissant le temps de résidence moyen des masses d'eau, l'estuaire peut être considéré comme un "raceway" dans lequel la population d'huîtres est soumise aux mêmes conditions de nutrition. Notons $C(t)$ la concentration énergétique de nourriture (joules par litre) dans la boîte, $C_0(t)$ la concentration en amont (limite) à l'instant t, V son volume (mètres cube) et F son débit (mètres cube par jour) d'entrée (ou de sortie), N_i le

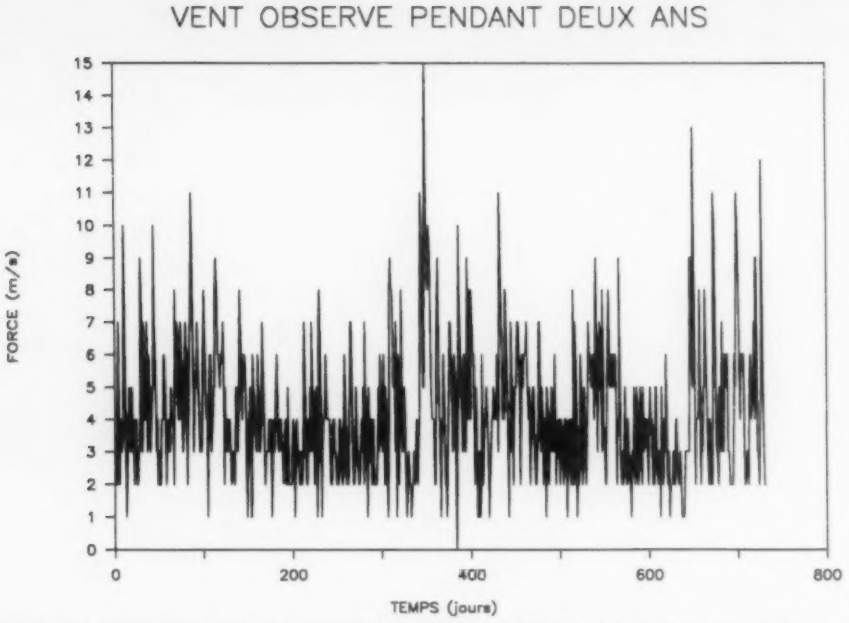

FIG. 3. Série observée de la force du vent en 1979–1980 introduite dans le modèle de reconstitution des séries temporelles des seston et PLG (source Météorologie Nationale). Le vent agit sur la concentration particulaire à la fois en modifiant le transport par les courants et en contribuant à la remise en suspension du fond.

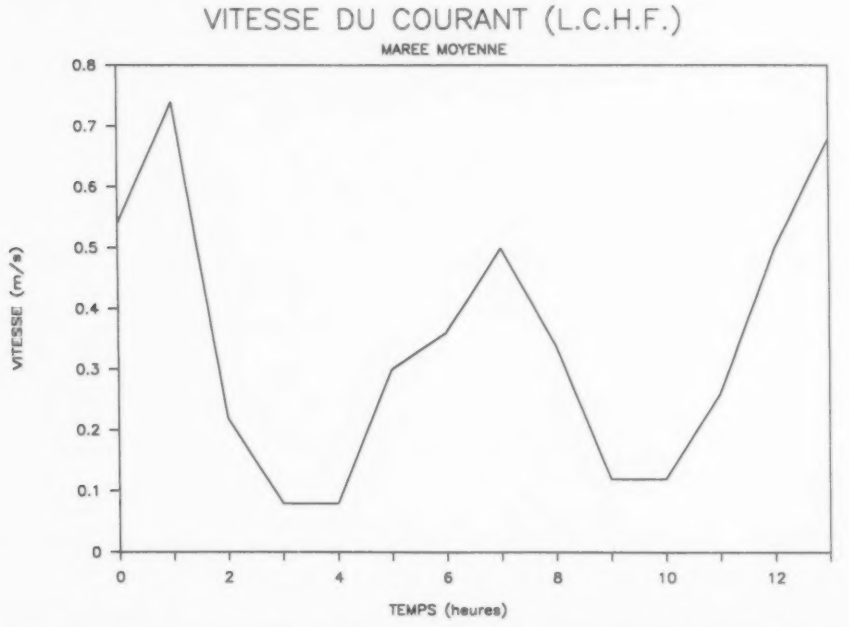

FIG. 4. Force du courant observé pendant un cycle de marée moyenne en partant de l'heure de basse mer (LCHF 1973).

nombre d'individus de la classe d'âge i ($i = 1, 2$), le système s'écrit

$$V\,dC/dt = F\cdot(C_0 - C) - \sum_i A_i\cdot N_i, \quad \text{équation de conservation}$$

$$dW_i/dt = (A_i - R_i)\cdot p_i\cdot \mathrm{TIM}, \quad \text{équation de croissance}$$

où A_i = assimilation (joules par heure), R_i = respiration (joules par heure) et W_i = poids (joules) individuels pour les classes 1 et 2 (cf. équation 1).

Pour simplifier, la donnée limite $C_0(t)$ sera représentée par la série utilisée dans le modèle énergétique. La variable $S(t)$ est

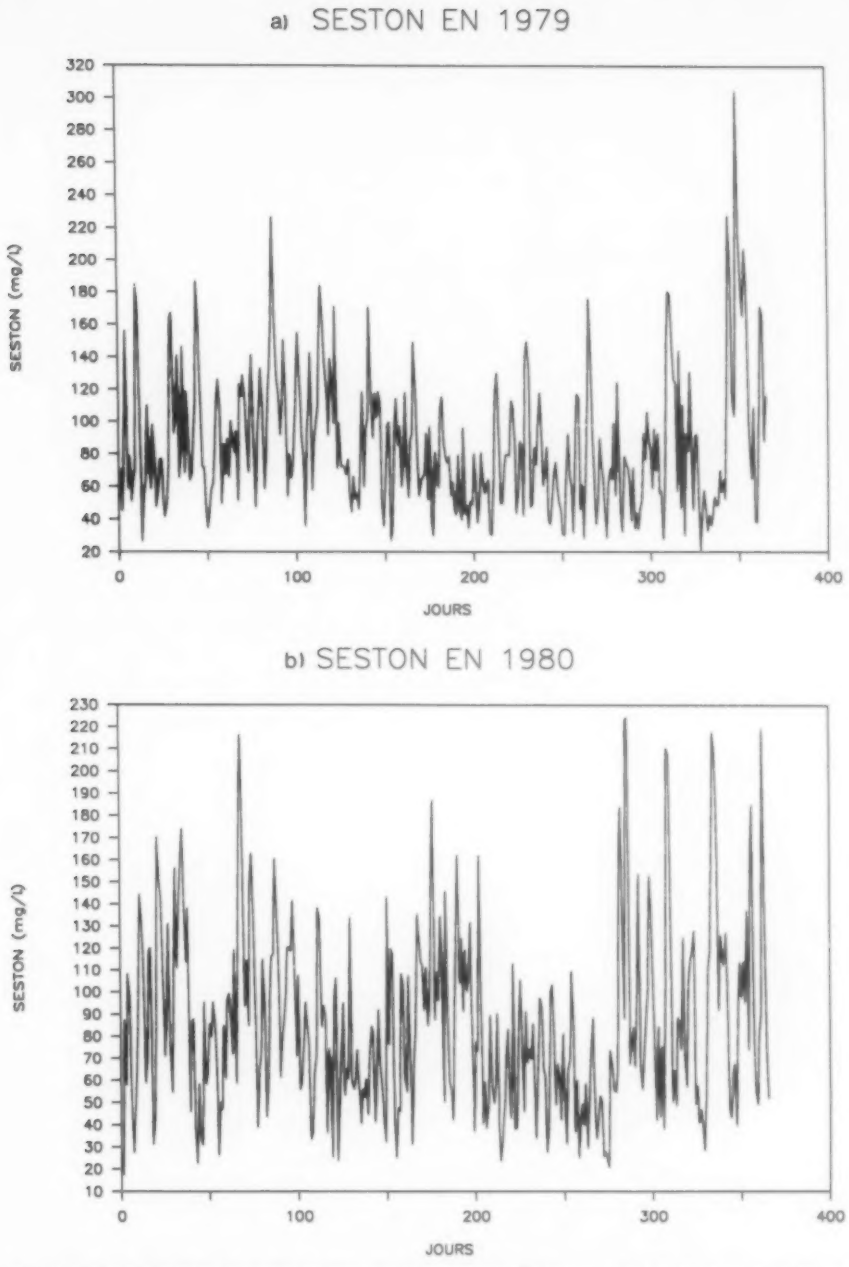

FIG. 5. Séries journalières simulées du seston et de la variable PLG (somme des équivalents énergétiques des concentrations en protides, glucides et lipides particulaires de la colonne d'eau) en 1979 et 1980 en prenant en compte la force du vent, la vitesse du courant et la hauteur d'eau. Ces simulations font ressortir l'évolution annuelle liée à la saisonnalité et la forte variabilité journalière due au vent. a) Seston en 1979; b) seston en 1980; c) PLG en 1979; d) PLG en 1980.

représentée par la série vue plus haut en supposant que les pseudofèces et fèces sont intégralement remis en suspension.

Le volume moyen du Bassin a été calculé d'après les sorties en hauteur d'eau d'un modèle courantologique réalisé par le LCHF (1979), selon un maillage rectangulaire pour deux coefficients de marée types. Le débit est le débit résiduel moyen estimé d'après les mesures et calculs effectués en morte-eau et vive-eau par le LCHF (1973). La croissance finale et le maximum de croissance (précédant la ponte) sont fonctions des effectifs N_i ($i = 1, 2$). Le stock d'huîtres, estimé par échantillonnage annuel depuis 1984, a été converti en effectifs pour les classes d'âge 1 et 2 ans (Bodoy *et al.* 1987). Des simulations sont effectuées avec différents niveaux d'effectifs de chaque classe d'âge. Trois types de sorties sont représentés graphique-

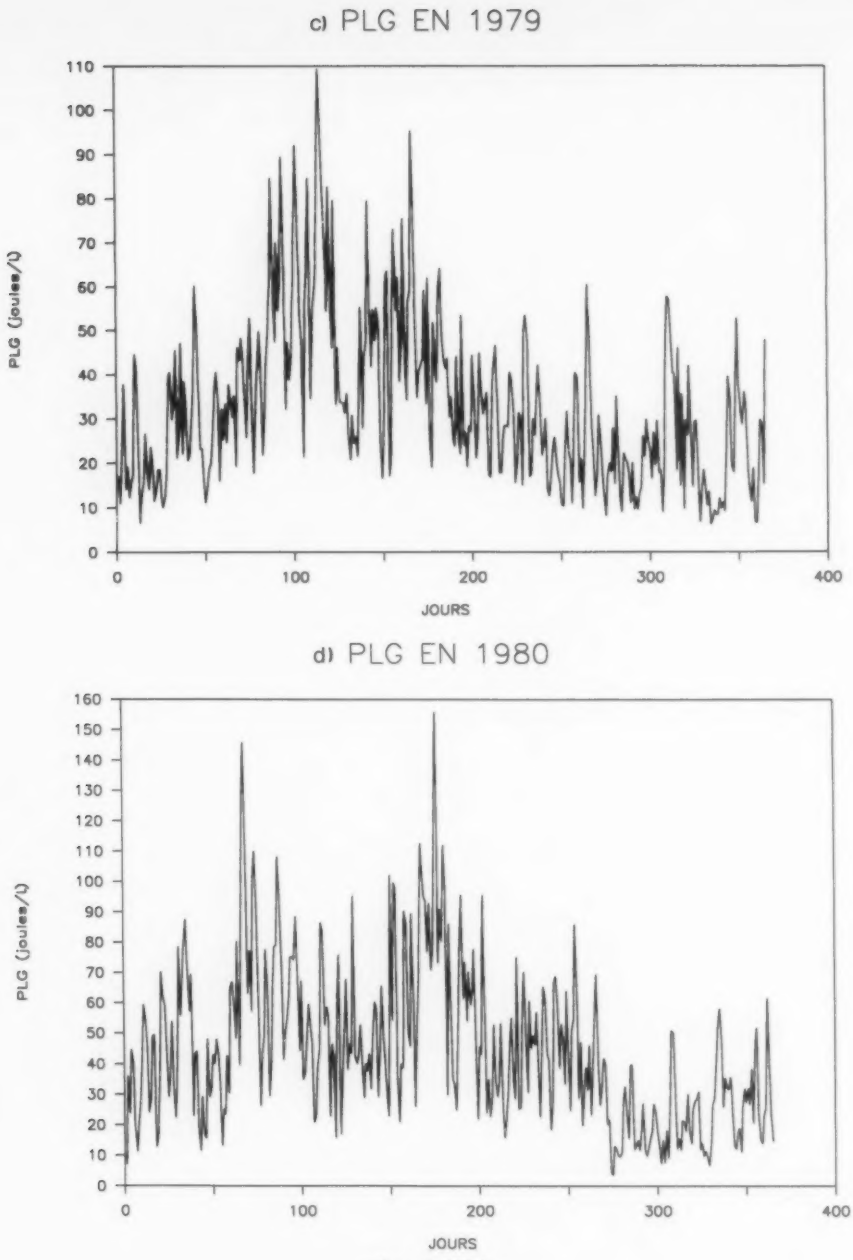

FIG. 5. (*Fin*)

ment afin d'illustrer la relation effectif/performance de croissance :

a) poids individuel moyen en fin de croissance.

b) production totale annuelle de la classe d'âge 1 (PT_1) et production totale des classes 1 et 2 (PT_2).

$$PT_1 = N_1 \cdot W_1$$
$$PT_2 = N_1 \cdot W_1 + N_2 \cdot (W_2 - W_1)$$

avec N_1 (resp. N_2) = effectif de la classe d'âge 1 (resp. 2) et W_1 (resp. 2) = poids final de la classe 1 (resp. 2).

c) proportion de nourriture potentielle assimilée en un an par la population.

$$PA = \frac{\sum_i \int_t N_i \cdot A_i(t)\, dt}{F \cdot \int_t C_0(t)\, dt}, \quad i = \text{classe d'âge.}$$

Résultats

Variabilité du milieu

L'ANOVA sur les différents facteurs de variabilité naturelle

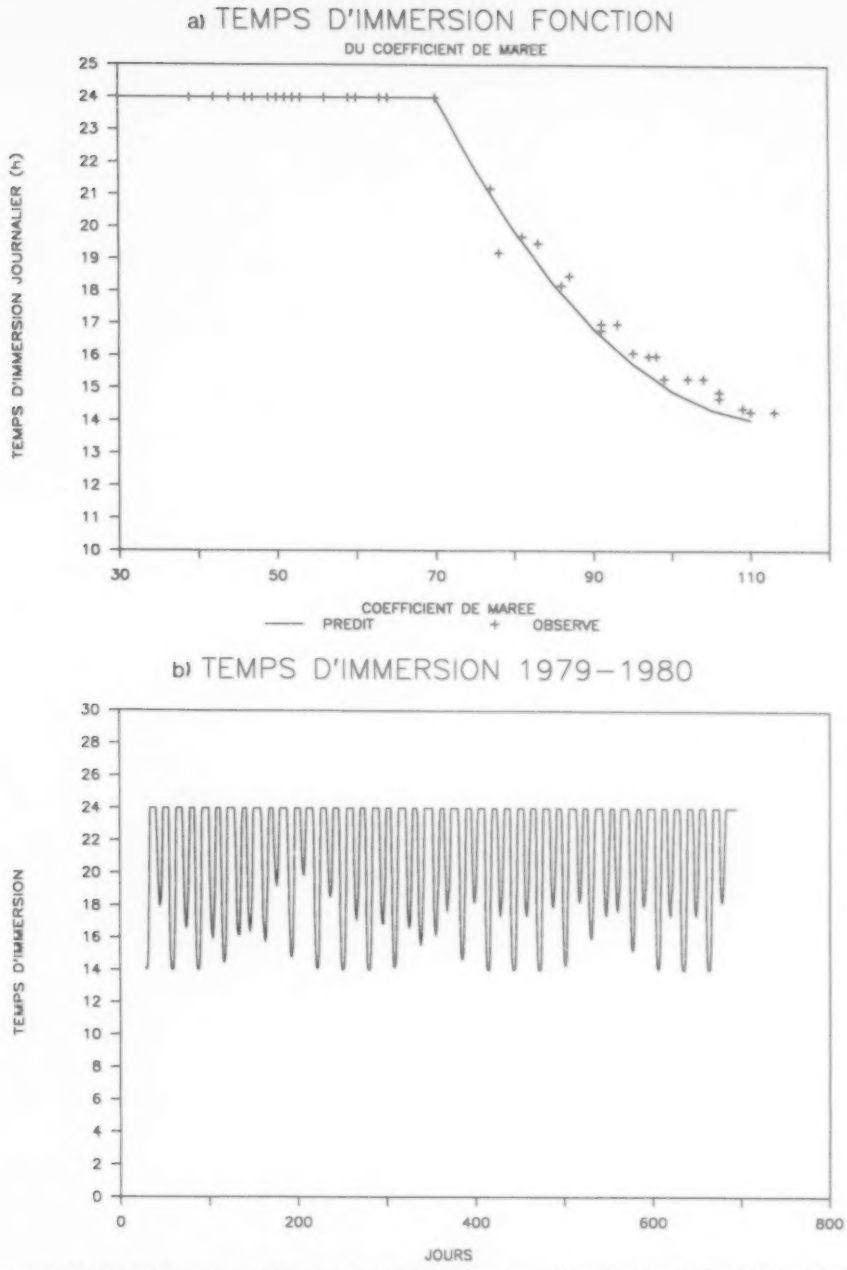

FIG. 6. Variables forçantes utilisées dans le modèle de bilan énergétique (années 1979 et 1980). a) Loi empirique du temps d'immersion en fonction du coefficient de marée (+, observations: — prédictions); b) Temps d'immersion sur 2 ans; c) température mesurée pendant 2 ans.

du seston (variable *S*) et de la variable PLG permet de hiérarchiser les contributions de chaque facteur à la variabilité observée. Quelles que soient l'année et la variable étudiée, il y a interaction entre la date et le coefficient de marée (probabilité $< 10^{-4}$). Afin d'étudier cette interaction, des ANOVA ont été réalisées sur les facteurs date et type de marée pour chaque modalité de hauteur de prélèvement. La représentation graphique des moyennes et intervalles de confiance montre une différence entre les types de marée pour les mois de février, mars et avril pour les deux variables étudiées en 1979 et 1980 (fig. 2). En général, cette différence est moins nette le reste de l'année. Il peut arriver qu'elle s'inverse lors de conditions météorologiques particulières (tempête en novembre 1980). On peut donc affirmer que le cycle morte eau-vive eau explique une part, fonction du temps mais importante, de la variabilité observée. Le bloom phytoplanctonique explique la tendance saisonnière. La combinaison des effets saisonnier et tidal et de leur interaction explique environ 80% de la

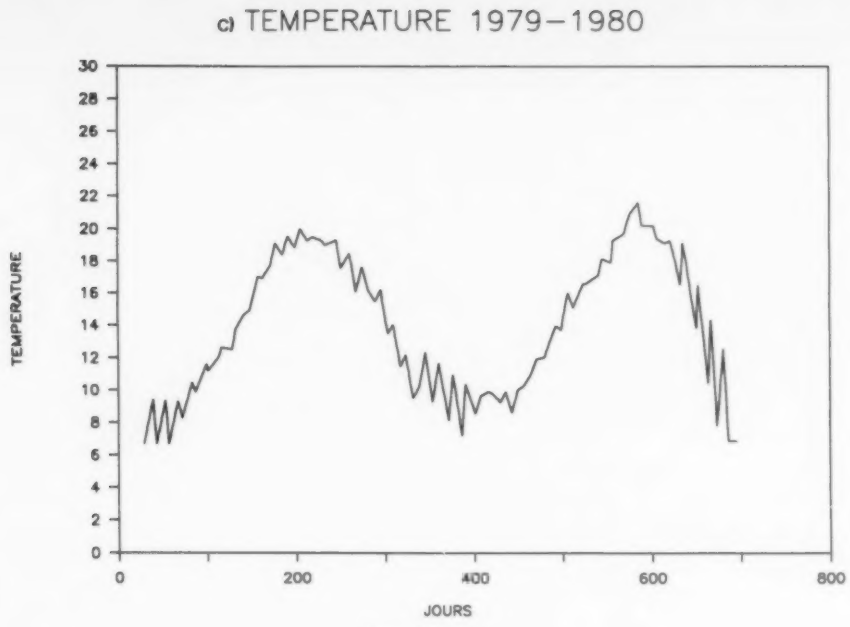

FIG. 6. (*Fin*)

variabilité totale (tableau 1). La variabilité résiduelle intègre l'erreur du modèle, la variabilité horaire.

Ces résultats justifient l'utilisation d'un modèle prédictif incluant principalement des fonctions périodiques correspondant aux sources majeures de fluctuations et un terme susceptible d'expliquer une partie de la variance résiduelle sous forme d'un processus stochastique. Courants, hauteurs d'eau et vent sont donc introduits dans un modèle mixte comprenant des termes linéaires et des effets multiplicatifs.

La sélection des variables les plus significatives dans l'explication des variables *S* (seston total) et PLG (somme des concentrations de protides, lipides et glucides converties en énergie) montre que les termes non linéaires jouent un rôle toujours négligeable (tableau 2). Par contre, un gain notable de signification est obtenu grâce au terme autorégressif. Le coefficient de détermination, qui mesure le pourcentage de variance expliquée, atteint ainsi 60 % pour le seston total. Pour la variable PLG, aucun facteur physique n'est retenu et le terme autorégressif explique à lui seul 62 % de la variance. Dans l'optique prédictive adoptée jusqu'à présent, il a semblé judicieux, bien que moins rigoureux, de calculer le cycle de la variable PLG à partir du cycle de la variable *S*, corrigé d'un facteur estimé mensuellement par la moyenne des rapports observés PLG/*S*.

La fonction de régression obtenue pour le seston total a été utilisée pour reconstituer le cycle annuel avec un pas de temps journalier en fonction du vent (fig. 3) et du courant (fig. 4). Initialisée par une valeur de seston total arbitraire, une série a été simulée sur les deux années 1979–1980. Les séries des moyennes journalières ont été ensuite calculées pour le seston total et la variable PLG (fig. 5). Les valeurs extrêmes de seston total (supérieures à 100 mg/L) sont mal reproduites. Elles correspondent à des phénomènes accidentels (tempêtes) à l'origine de remises en suspension massives et de courte durée. Leur probabilité d'occurrence est faible et peut être considérée comme dépendant de la saison. Elle est ainsi plus forte en hiver et à la fin de l'automne, périodes correspondant à un amaigrissement relativement faible des huîtres. Ces accidents météorologiques apparaissent parfois en période de croissance mais leur courte durée, liée à une resédimentation rapide, permet de tempérer leur influence sur la croissance des huîtres.

Modèle énergétique

La fig. 6 montre la loi du temps d'immersion en fonction du coefficient de marée et l'évolution du temps d'immersion qui en a été déduite au cours du temps. La variation journalière de la température sur 2 ans est représentée sur la fig. 6c. L'optimisation par la méthode du simplex permet d'estimer les paramètres inconnus du modèle de croissance. La comparaison des poids secs simulés et observés (fig. 7) après calage sert à valider le modèle. La pente de la régression est voisine de 1 (à 5 %) et l'ordonnée à l'origine de 0 (à 5 %). Le nombre de degrés de liberté est égal à $N-p-1 = 20$ (N = nombre d'observations = 26, p = nombre de paramètres estimés grâce aux mêmes observations = 5).

Le modèle monoboite théorique illustre la relation effectif-performance de croissance. Le temps de résidence est égal au rapport V/F. Le volume moyen V prend la valeur 675×10^6 m^3. Le débit F est de l'ordre de 200×10^6 m^3/jour en coefficient de vive-eau et 100×10^6 m^3/jour en morte-eau. Le temps de résidence moyen adopté est donc de 4,5 jours. Vingt simulations ont été réalisées pour des effectifs variables en prenant comme hypothèse que la quantité de nourriture disponible $C_0(t)$ à la limite du modèle est indépendante de l'état du stock. Les productions, les poids en fin de croissance et les proportions de nourriture assimilée sont reportés en fonction de l'effectif (fig. 8). La pente dY/dX de chaque courbe permet de calculer la sensibilité s de la variable expliquée à une variation de l'effectif au voisinage de l'effectif actuel X_0 :

$$s = \frac{X_0}{Y_0} \cdot \frac{dY}{dX}$$

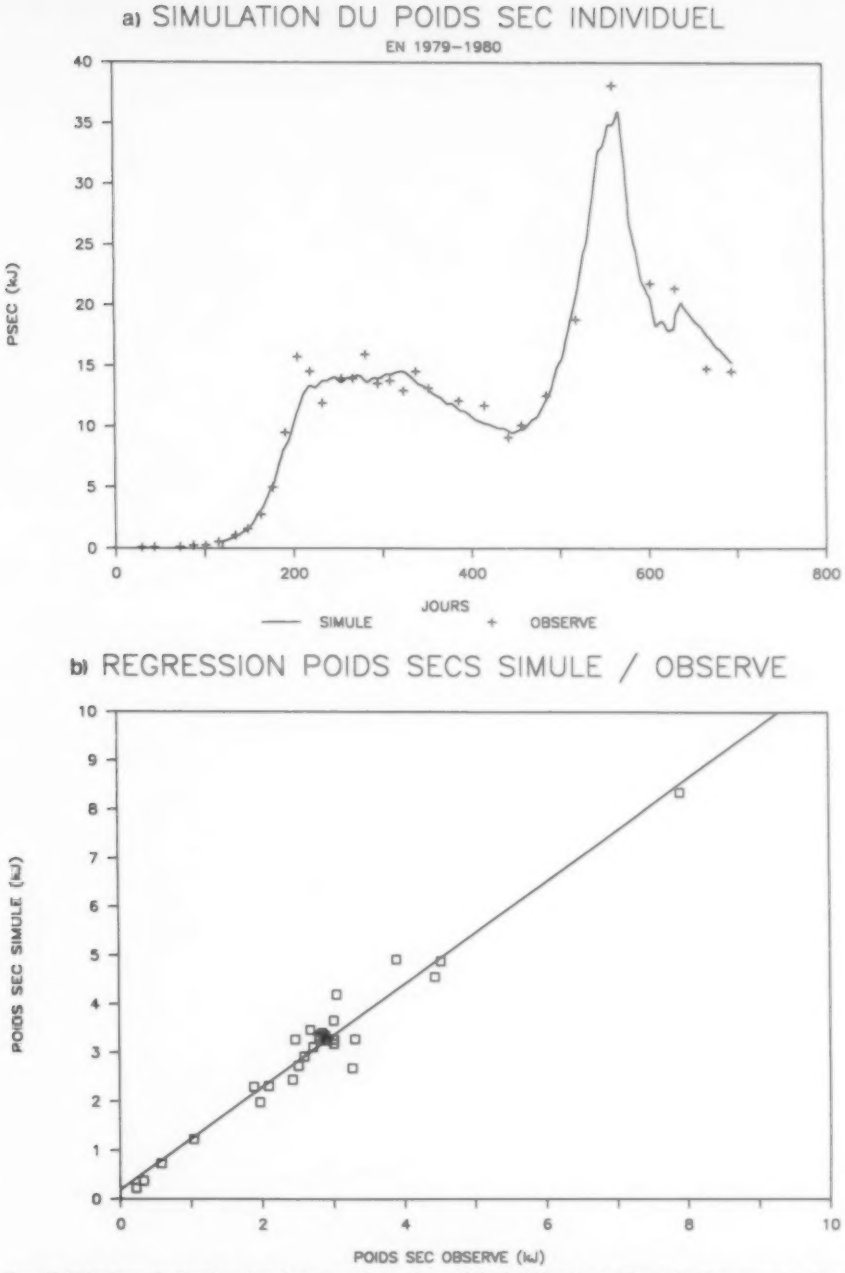

FIG. 7. Résultats de l'estimation des paramètres du modèle de bilan énergétique de *Crassostrea gigas*. a) Simulation pendant 2 ans du poids sec individuel moyen converti en énergie (—) et comparaison avec les valeurs observées (+). La croissance au début de la deuxième année est compensée par une ponte estivale très importante, suivi d'un amaigrissement automnal et hivernal : $F_1 = 24{,}32$, $F_2 = 0{,}602$, $S_0 = 12{,}22$, $R_0 = 1{,}746$, $R_1 = 1{,}3$, $R_2 = 0{,}1$, $T_0 = 9{,}14$, $T_1 = 23{,}80$. b) Régression entre les valeurs simulées et observées du poids sec individuel moyen : $Y = aX + b$, $N = 26$, $p = 5$; $a = 1{,}055$, $\sigma(a) = 0{,}047$; $b = 0{,}19$, $\sigma(b) = 0{,}15$.

où Y_0 est la valeur de la variable expliquée pour l'effectif X_0. La sensibilité de la proportion assimilée est égale à 0,78, très proche des sensibilités des productions PT_1 et PT_2 (0,83 et 0,84 respectivement). Pour les poids en fin de croissance, les sensibilités valent $-0{,}16$ pour les deux classes d'âge. Une augmentation du stock se traduit donc par une diminution sensible des performances de croissance.

Discussion

Le présent modèle permet de présenter le problème de capacité trophique d'un site côtier consacré à la conchyliculture. Par

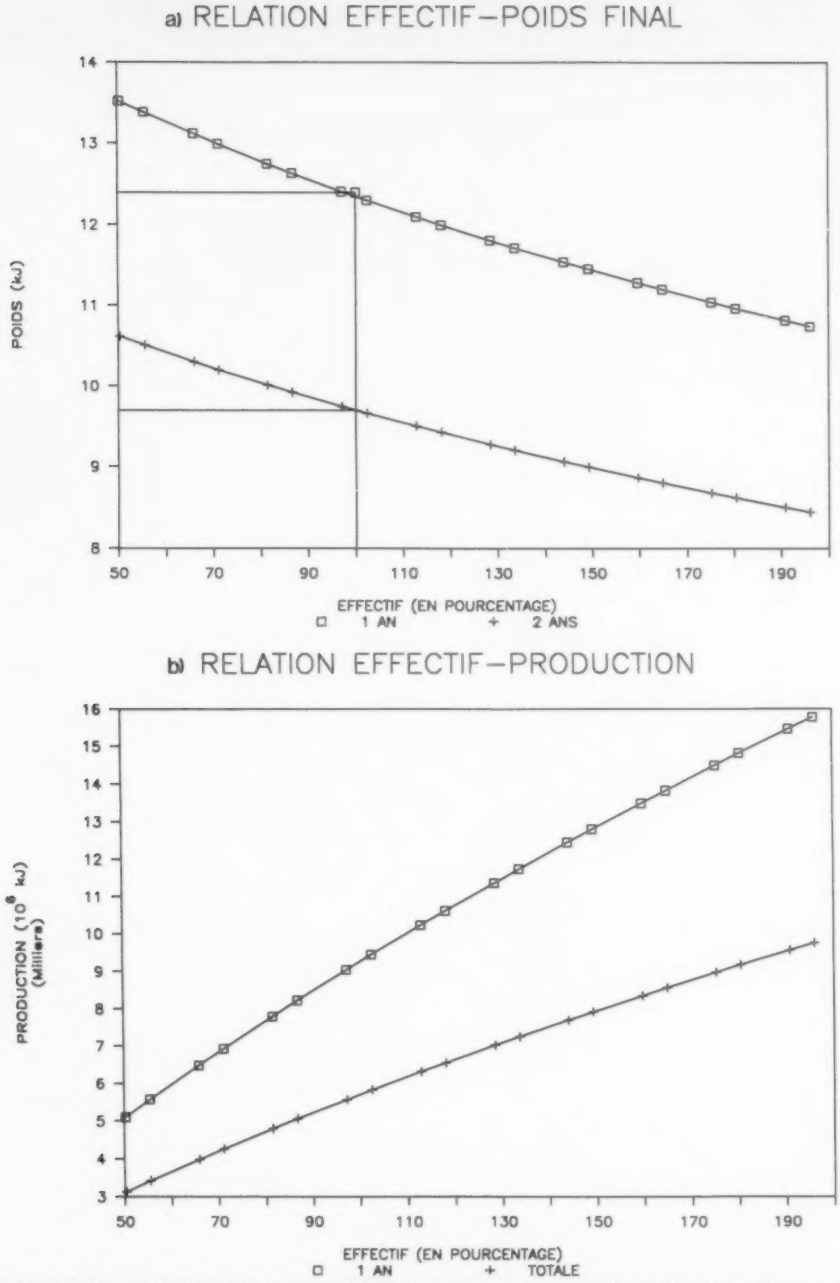

FIG. 8. Influence de l'effectif de la population de *Crassostrea gigas* sur les résultats de croissance en utilisant un modèle monoboite. La variation de l'effectif est exprimée en pourcentage de l'effectif observé en 1986. La compétition pour la nourriture aboutit à une diminution des performances individuelles de croissance lorsque la quantité d'huîtres augmente (a). Si le nombre d'huîtres est multiplié par 4, la production totale (b) et le pourcentage de nourriture utilisée pour la croissance (c) sont ainsi multipliés par un facteur 3. a) Poids sec individuel converti en énergie du bout de 1 an (□) et 2 ans de croissance (+); b) production pour la classe d'âge 1 (□) et la population totale (+); c) pourcentage de nourriture potentielle asimilée par la population par rapport aux flux entrant dans le bassin.

rapport aux études existantes (Incze *et al.* 1981; Lutz 1980; Verhagen 1982), il donne une idée de la compétition intraspécifique au niveau macroscopique, bien que certains auteurs (Peterson et Black 1987) insistent sur l'existence d'une compétition au niveau local. L'écriture d'une équation de bilan énergétique permet d'aller plus loin que les études plus classiques des relations trophiques dont le but est d'étudier les performances de croissance en fonction des paramètres du milieu

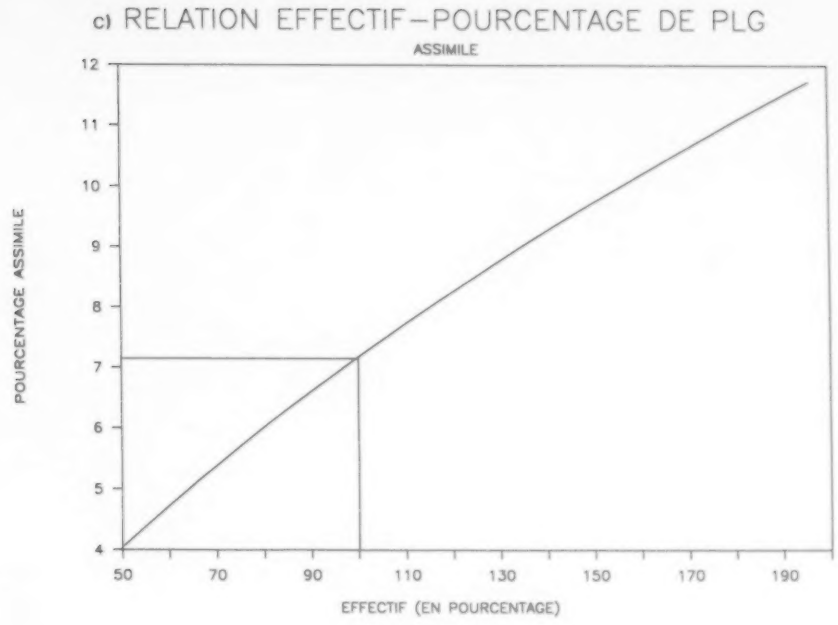

FIG. 8. (*Fin*)

(Goulletquer et Bacher 1989). Ce modèle indique ainsi que, même si le pourcentage d'énergie assimilée paraît faible (environ 7 %), la déplétion de nourriture est déjà suffisante pour qu'une augmentation des effectifs se traduise par une perte de croissance équivalente. La sensibilité de la production, inférieure à 1, montre le ralentissement de l'augmentation de production résultant de l'accroissement du stock. La concavité de la courbe de production illustre la non-linéarité de la réponse de la population à une variation des effectifs. C'est la définition même d'un milieu dont la nourriture est limitante. Cette conclusion remet ainsi en cause les calculs de bilan et de pourcentage d'énergie utilisée au niveau local sans tenir compte du temps de résidence des masses d'eau (Héral *et al.* 1983a). C'est donc bien le phénomène de transport particulaire qui détermine la capacité trophique du Bassin. Il parait ainsi probable que des différences spatiales de croissance peuvent être également expliquées par l'appauvrissement des masses d'eau transitant dans le bassin, au fur et à mesure de leur utilisation par les filtreurs. L'homogénéisation des masses d'eau dans les estuaires macrotidaux montre cependant qu'il n'y a pas déplétion de nourriture à une échelle spatiale inférieure au kilomètre. L'échelle spatiale compatible avec la perte de nourriture due à la consommation dépend finalement de l'amplitude de l'excursion de marée, largement responsable du confinement des masses d'eau. Le couplage d'un modèle physique de transport particulaire et du modèle de croissance doit permettre d'approfondir l'étude du lien stock-performance de croissance. Il est déjà clair qu'il existe un gradient de croissance nord-sud (A. Bodoy, comm. pers.) et que ce gradient est corrélé à l'écoulement résiduel des masses d'eau transitant dans le bassin (Bacher 1989). La variabilité spatiale des performances de croissance ne peut être approchée que par un modèle associant croissance et nourriture disponible selon un découpage spatial du Bassin. De cette étape résulte la possibilité d'une gestion des stocks d'huîtres et de leur répartition en fonction d'un objectif de croissance à déterminer.

Plusieurs types de problèmes ont été rencontrés au niveau de la modélisation. La difficulté à trouver des données bibliographiques quantifiant les réponses des différentes fonctions d'ingestion et d'assimilation constitue un obstacle certain à la robustesse du modèle. Il est clairement démontré, par des suivis *in situ* et des méthodes de régression (Brown 1988), que température et nourriture contrôlent le bilan de croissance. Cependant, la formulation de la réponse des mécanismes de filtration, ingestion, assimilation et respiration en fonction de ces variables ne fait pas l'unanimité. Rodhouse et O'Kelly (1981) observent ainsi une variation linéaire du taux de filtration de *Crassostrea gigas* en fonction de la température sans influence de la concentration particulaire. Gerdès (1983) au contraire constate une régulation de la filtration en fonction de la concentration algale. Une autre difficulté provient de ce que les expériences de nutrition (Epifanio 1979; Urban *et al.* 1983) ne visent pas à reproduire les conditions naturelles caractérisées par une composition et une proportion variable de matière organique particulaire et une turbidité relativement élevée. Aussi, la calibration réalisée sur les données environnementales ne permet pas de généraliser le modèle à un site trop différent de celui étudié. Le domaine de validité du modèle ne s'étend ainsi qu'aux sites de caractéristiques hydrobiologiques comparables. Cependant, le bassin de Marennes-Oléron est un système macrotidal marqué par des cycles de marée très accentués. Malgré un temps de résidence moyen de 5 jours, l'amplitude de l'excursion de marée et l'existence d'estrans de surface importante justifient l'idée de masses d'eau homogènes à l'échelle macroscopique (quelques kilomètres). La situation centrale du point d'étude choisi garantit sa représentativité de l'ensemble du Bassin (Héral *et al.* 1983b). Il reste néanmoins souhaitable d'intégrer dans cette démarche les résultats de plans d'expérience dans lesquels les paramètres sont contrôlés (Raillard, en prép.).

La sensibilité des séries temporelles de seston et de PLG aux accidents météorologiques, à l'échelle de temps des méca-

nismes biologiques, peut être expliquée par l'influence du vent sur le transport particulaire et la remise en suspension du sédiment. L'importance de ces phénomènes et la difficulté de reconstituer la série de la nourriture potentielle mesurée par la somme des variables protides, lipides et glucides montrent l'effort à fournir en matière d'échantillonnage en milieu estuarien. La différence entre les deux variables expliquées est à rechercher vraisemblablement dans l'existence de cycles organiques saisonniers, identifiés par Héral *et al.* (1983b) sous la forme d'apports phytoplanctoniques ou détritiques d'origine océanique (été) ou continentale (hiver). Dans la perspective du couplage d'un modèle de transport et du modèle de bilan énergétique, le besoin de séries aux limites du modèle spatial renforce l'idée d'un accroissement de l'effort d'échantillonnage. La période hivernale expliquant peu les variations de croissance, l'effort en question est à moduler selon les saisons.

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Role of Fat Stores in the Maturation of Male Atlantic Salmon (*Salmo salar*) Parr

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Rowe, D. K., J. E. Thorpe, and A. M. Shanks. 1991. Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. Can. J. Fish. Aquat. Sci. 48: 405–413.

We provide evidence that in male Atlantic salmon (*Salmo salar*) parr, maturation is suppressed when mesenteric fat fails to exceed an undefined level by May. In nonmaturing parr the postwinter increase in total lipids began in May, while the mesenteric store started filling in June. However, in male parr which would have matured, total lipids started increasing a month earlier, in April, and the mesenteric store started filling in May. Consequently, maturing male parr had significantly more mesenteric fat than nonmaturing fish by June. Mesenteric fat is needed for maturation. Levels continued to increase in nonmaturing parr during autumn months, but had declined in maturing parr by September. This depletion of mesenteric fat in maturing males coincided with increases in the gonadosomatic index from 0.05 to 10% and with reductions in both feeding and growth. Fasting during spring months delayed increases in total lipids and fat accumulation into the mesenteric store until June and suppressed maturation rates of male parr. The internal decision to suppress maturation is therefore dependent on mesenteric fat levels increasing in May. However, this requires the prior replenishment of other stores in April. A model is proposed to explain the physiological link between fat accumulation during spring and the initiation of maturation.

Les auteurs prouvent que dans les tacons mâles de saumon de l'Atlantique la maturation est supprimée lorsque, au mois de mai, la teneur en graisse mésentérique ne dépasse pas un niveau indéterminé. Chez les tacons qui ne montrent pas de signes de maturation, l'augmentation post-hivernale de la teneur totale en lipides commence en mai, tandis que la réserve de graisse mésentérique commence à se remplir en juin. Toutefois, chez les tacons mâles qui auraient atteint la maturation, la teneur totale en lipides commence à augmenter un mois plus tôt, soit en avril, et la réserve en graisse mésentérique commence à s'accumuler en mai. En conséquence, dès juin, les tacons mâles en phase de maturation montrent une teneur en graisse mésentérique nettement plus élevée que les poissons qui ne montrent pas de signes de maturation. La graisse mésentérique est nécessaire à la maturation. Chez ceux-ci, les teneurs continuent à augmenter pendant l'automne, mais elles diminuent dès septembre chez les tacons en maturation. Cette diminution de la teneur en graisse mésentérique chez les mâles en maturation coïncide avec une augmentation de l'indice gonadosomatique de 0,05 à 10 % et à des diminutions des taux d'alimentation et de croissance. Le jeûne printanier a retardé l'augmentation de la teneur totale en lipides et l'accumulation de graisse dans la réserve mésentérique jusqu'en juin, en plus d'éliminer la maturation chez les tacons mâles. L'élimination de la maturation dépend donc de l'augmentation de la teneur en graisse mésentérique en mai. Toutefois, ce processus interne exige la régénération antérieure des autres réserves en avril. Les auteurs présentent un modèle expliquant le lien physiologique entre l'accumulation de graisse au printemps et le début de la maturation.

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A number of studies have indicated that the size of lipid stores is associated with the initiation of maturation in salmonids. Shulman (1974), in an extensive review of the literature on this topic, found evidence to support the concept of a minimum fat content before maturation is initiated in a number of species. More specifically, Reshetnikov et al. (1970) found that the onset of maturation in whitefish (*Coregonus lavaretus*) was connected with the attainment of a level of fat reserves specific to each population. Similarly, Barbour (1985) measured seasonal energy contents of Arctic char (*Salvelinus alpinus*) and hypothesized that the onset of maturation was triggered by the amount of energy they will have at their disposal during maturation. Thurow (1966) proposed that Atlantic salmon (*Salmo salar*), returning from the sea, needed a minimum fat content of 12% in spring if they were likely to spawn during the following autumn. Thorpe (1986) also indicated that spring was a critical time, but he suggested that the rate of acquisition of energetic reserves could be more important than attaining a specific level.

Male parr of Atlantic salmon frequently mature at a small size in freshwater (Thorpe 1986), and there is considerable interest in the causes and consequences of this early maturity (Meerburg 1986). Rowe and Thorpe (1990a) investigated the effect of growth on the maturation of male parr. They found that spring growth opportunity was important for the control of

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maturation, but there was no relationship between monthly specific growth rates and maturation (Rowe and Thorpe 1990b). However, maturing parr did show greater increases in condition factor compared with nonmaturing fish during April. As condition factor is directly related to fat content of Atlantic salmon parr (Pinder and Eales 1969), it was hypothesized that spring growth in fat reserves, rather than in body size, was linked with the control of maturation in the male Atlantic salmon parr.

Fish contain a number of fat reserves which could be involved in the control of maturation. Although the liver is the major fat store in many demersal marine fish, muscle fat is of greater importance in pelagic species (Cowey and Sargent 1972), and mesenteric fat is a major store in salmonids (Henderson and Sargent 1981). We observed extensive deposits of fat on the mesenteric tissues of maturing male Atlantic salmon parr during June and July. However, Leyzerovich (1973) found that, after July, maturing male parr had less mesenteric fat than immature ones. He suggested that differences in the dynamics of mesenteric fat may be involved in the maturation process.

The aims of this study were firstly to compare the seasonal cycles of depletion and replenishment for total lipids and mesenteric fat in maturing and nonmaturing parr and secondly to determine the role of mesenteric fat during maturation. We then tested the hypotheses that (1) total fat content and mesenteric fat levels increase more rapidly in maturing males than in nonmaturing males during spring months and (2) fat contents of maturing male parr are greater than those of nonmaturing males before gonadal recrudescence begins. Finally, as food restriction during spring months suppresses maturation (Rowe and Thorpe 1990a), we determined the effect of fasting on seasonal fat dynamics.

Methods

Seasonal Changes in Fat Levels and Effects of Fasting

Experiments to determine seasonal changes in fat content, and effects of fasting on these, were combined. Details of the experimental conditions are given in Rowe and Thorpe (1990a). Each month, from January to June 1988, random samples of twenty 1-yr-old salmon parr were removed from both an experimental (fasted on alternate weeks over the preceding 2 mo) and control (not fasted) group for lipid analysis. All groups were sampled for total fat content in July. Each fish was weighed, measured, and sexed. Gonads and mesenteric fat were then weighed and lipid content of the entire fish determined using Hanson and Olley's (1963) method.

Seasonal changes in fat levels of male and female parr were determined from the monthly samples of control fish. Virtually all males (93%) in control groups matured, but no females did. There was no difference in mesenteric fat levels (this study) and total lipids (Saunders and Sreedharan 1977; Koch and Bergstrom 1978) between nonmaturing male and female Atlantic salmon parr. The fat levels in female parr therefore reflected those in nonmaturing male parr.

Maturing male parr were generally larger than nonmaturing parr and can be expected to have higher fat contents. Total lipid and mesenteric fat weights were therefore expressed as a percentage of body weight. As the sizes of fish in the fasted versus control groups for a given month varied, analysis of covariance (ANCOVA) of log-transformed values was used to determine differences in fat levels between control and experimental groups.

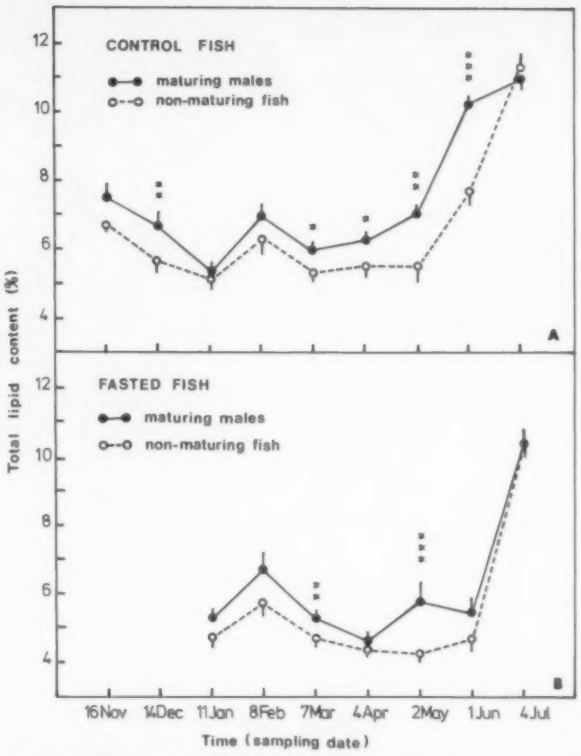

FIG. 1. Differences in monthly fat levels (mean ± SE) between maturing males and nonmaturing fish for (A) control groups of 1+ salmon parr and (B) groups fasted over the previous 2 mo. Asterisks indicate significant differences between maturing and nonmaturing fish: $***p < 0.001$, $**p < 0.05$, $*p < 0.10$.

Role of Mesenteric Fat in Maturing Parr

Approximately one hundred 1-yr-old sibling salmon parr were sampled in July, September, and November 1987. Fish were weighed and sexed and the gonads extracted and weighed to the nearest 0.0001 g. Fatty tissue was dissected away from viscera and also weighed to the nearest 0.0001 g. Weights of mesenteric fat were expressed as a mesenteric fat index (MFI):

$$\text{MFI} = 100 \cdot W_1/W_2$$

where W_1 is the wet weight (grams) of mesenteric fatty tissue and W_2 is the wet weight (grams) of fish.

MFI's were plotted against gonadosomatic indices (GSI) to determine effects of maturation on mesenteric fat levels.

Results

Maturing males contained higher proportions of total lipids than nonmaturing fish (Fig. 1A) and had higher MFI's (Fig. 2A). There was no difference ($p > 0.10$, Mann–Whitney U test) in the mean lipid content of mesenteric fatty tissue between maturing and nonmaturing fish. Values ranged from 86 to 98% (median value 90.9 for maturing males and 92.4 for other fish). This store therefore constituted a major component of total lipids in all fish, accounting for up to 45% of total fat content in maturing males.

Seasonal Variations in Lipid Stores

There was a positive correlation between mesenteric lipid and total fat content for all months (Table 1). Despite this the seasonal pattern of depletion and replenishment of total fat and mesenteric fat differred.

Apart from an increase in January, total fat levels declined in both maturing and nonmaturing parr between 16 November and 7 March (Fig. 1). The apparent increase in January was due more to a drop in weight of parr at this time (Rowe and Thorpe 1990b) than to an actual increase in fat. Total fat contents of maturing males then began to increase in April, a month earlier than in nonmaturing fish (Fig. 1A). Levels reached 10% of body weight (30% higher than in nonmaturing fish) by the end of May. However, the rate at which total body lipids increased declined during June for maturing males relative to nonmaturing fish. As a consequence, there was no significant difference in total fat levels between maturing males and nonmaturing fish by July.

This seasonal pattern of depletion and replenishment of total fat also occurred in fasted fish (Fig. 1B). However, replenishment of total lipids during spring was delayed until June.

Mesenteric fat levels also varied seasonally (Fig. 2A), but changes were delayed relative to total body lipid levels. Mesenteric fat levels remained relatively constant between 16 November and 8 February, but had declined by 7 March. By April, the mean MFI of maturing males was significantly higher than for nonmaturing fish, due mainly to the continuing decline in mesenteric fat levels of the nonmaturing fish. MFI's did not increase substantially until May in maturing fish and June in nonmaturing fish. The rate of increase in mesenteric fat levels declined for maturing males during June.

Mesenteric fat levels of the fasted fish also declined during winter months (Fig. 2B). Spring increases were delayed until June, as occurred for nonmaturing fish in control groups.

Changes in Mesenteric Fat Indices (MFI's) during Maturation

Changes in GSI were used as a measure of maturation status. Although fish size increased between July and November (from modal fork length of 87 to 118 mm) the GSI of female fish remained constant throughout this time (Fig. 3). In comparison, GSI's of male fish were separated into two distinct groups by September. Those with GSI's below 0.05% were classified as immature, as GSI's of all male parr in the stock population were less than 0.05% in January. Those with GSI's above 0.05% were assumed to be maturing. As a relatively constant proportion (82–84%) of male parr were maturing in July, September, and November, gonadal recrudescence had started in virtually all maturing males by mid-July.

At this time the GSI's of maturing males ranged from 0.07 to 3.5%, but by September they averaged 10% (range 4.5–12%) (Fig. 4). MFI's of maturing males were positively correlated with GSI values in July ($r = +0.70$). By September, however, MFI's were negatively correlated with GSI ($r = -0.61$).

There was no significant difference in MFI between nonmaturing males and females in July, September, or November (Fig. 5). However, MFI's of maturing males were significantly greater ($p < 0.01$, Mann–Whitney U test) than those of nonmaturing fish in July. MFI's of the maturing males then declined between July and September whereas in females and nonmaturing males they increased. As a consequence, MFI's of nonmaturing fish exceeded those of maturing males by September.

Effect of Fasting on Lipid Stores

Significant differences occurred in fat content between fasted and seasonal control groups (ANCOVA, Table 2), but there was also a significant interaction component. This indicated that the magnitude of these differences varied with time. The overall mean weight of fish sampled was 3.8 g, so regressions of fat weight on body weight, calculated using a pooled slope of 1.232 (SE = 0.0247), were used to estimate fat contents of a standard sized (3.8 g) fish. The resulting "fitted" fat values show this seasonal variation in fat content between control and food-restricted groups (Fig. 6).

Fat contents of fish fasted between November and January were no different from controls. However, fasting from February until May inclusive suppressed fat levels relative to controls. The greatest effect followed fasting during April and May (i.e. for fish sampled 1 June). Because of the seasonal pattern of depletion and deposition in fat, the main effect of fasting was to delay the spring replenishment of fat reserves by approximately 1 mo.

Although fasting between April and May delayed replenishment of lipid stores until June, fasting between May and June apparently had no effect on fat levels. This may have been because lipid levels of fish sampled at the end of June were measured 8 d after the resumption of feeding, instead of the usual 2–3 d. Fat deposition was particularly rapid during June, even in food-restricted fish, and fat stores may have increased significantly in 1 wk, obliterating effects of fasting.

TABLE 1. Regressions of mesenteric lipid (g) on total lipid (g) for parr from the control populations (no food restriction) and experimental populations (starved during alternate weeks of the previous 2 mo).

	Control populations			Experimental populations		
Date	Correlation (r)	Intercept (a)	Slope (b)	Correlation (r)	Intercept (a)	Slope (b)
16 Nov.	0.92	−2.14	0.07	—	—	—
14 Dec.	0.96	−1.85	0.06	—	—	—
11 Jan.	0.98	−1.73	0.06	0.94	−1.74	0.05
8 Feb.	0.98	−1.73	0.07	0.97	−1.93	0.06
7 Mar.	0.81	−2.22	0.06	0.81	−2.41	0.06
4 Apr.	0.98	−2.17	0.06	0.94	−2.77	0.04
2 May	0.94	−2.51	0.08	0.96	−2.10	0.07
1 June	0.96	−2.39	0.13	0.91	−2.84	0.12
4 July	0.89	−1.72	0.50	0.97	−2.10	0.20

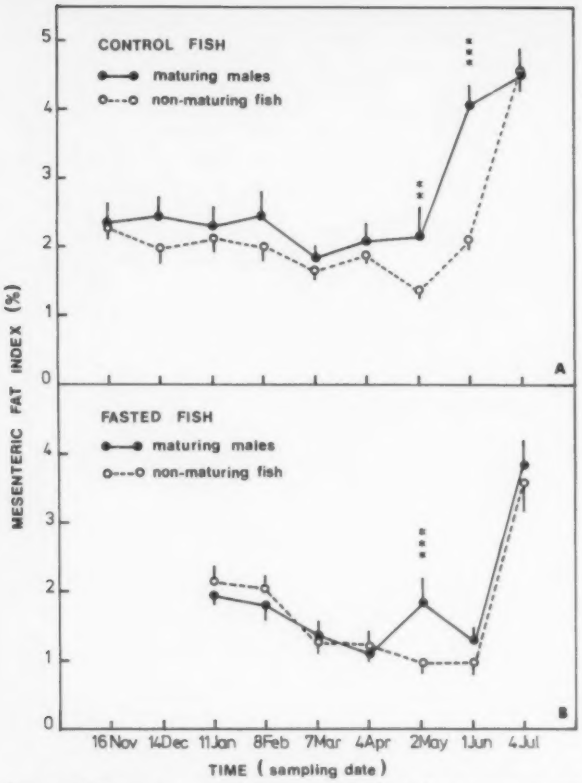

FIG. 2. Differences in monthly mesenteric fat indices (mean ± SE) between maturing males and nonmaturing fish for (A) control groups of 1+ salmon parr and (B) groups fasted over the previous 2 mo. Asterisks indicate significant differences between maturing and nonmaturing fish: $***p < 0.001$, $**p < 0.05$, $*p < 0.10$.

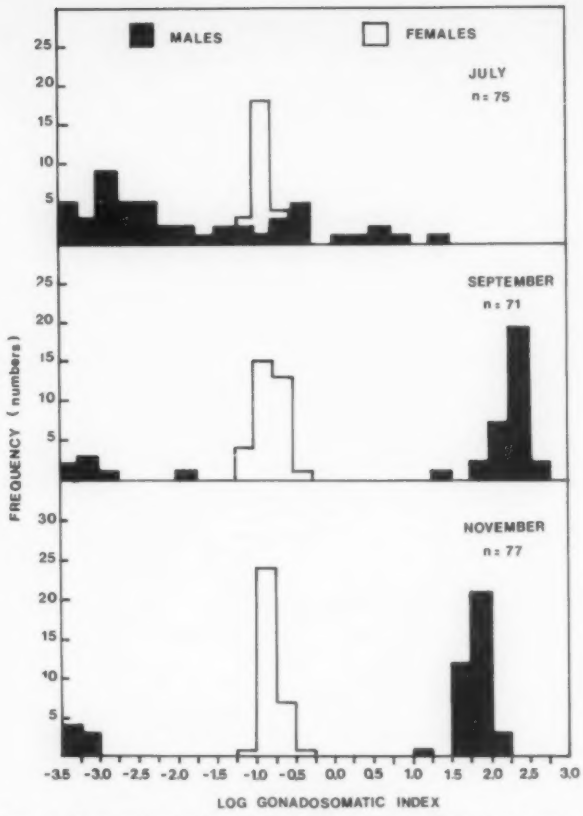

FIG. 3. Changes in the frequency distribution of log GSI's for male 1+ salmon parr relative to females between July and November 1987.

Discussion and Conclusions

Differences in Fat Levels between Maturing and Nonmaturing Fish

The null hypotheses that there is no difference in (1) total lipid and mesenteric fat levels or (2) fat accumulation rates between maturing males and nonmaturing males during spring and early summer months when gonad growth beyond the primary spermatogonia stage begins were both rejected. Increases in GSI past 3% are associated with advanced stages of maturation in male Atlantic salmon parr, notably the formation of primary spermatocytes (Murphy 1980). Before this stage was reached (July), maturing male parr had significantly higher levels of mesenteric fat compared with nonmaturing fish (Fig. 5).

Furthermore, the positive correlation between MFI and GSI in July indicates that the maturing males with relatively high fat levels had either started to mature earlier or were maturing at a faster rate than the maturing males with lower mesenteric fat levels. Although mesenteric fat levels of maturing parr were higher than those of nonmaturing fish between January and June, the difference was greatest at the end of May (Fig. 2A).

Seasonality of Fat Deposition and Effects of Fasting

The implication that maturing males have faster rates of fat deposition in April and May disguises the crucial importance of timing for replenishment of both total lipids and the mesenteric fat store after winter.

During winter, selective depletion of fat stores occurred. Mesenteric fat was reduced only after total body lipids were already substantially depleted. Then, in spring, replenishment of the mesenteric store was delayed until after total body lipids (hence nonmesenteric stores) were already increasing. As a consequence, mesenteric fat levels did not increase in maturing fish until May, with the mean MFI increasing by nearly 100% to reach 4% of body weight at this time (Fig. 2A).

In nonmaturing parr, mesenteric fat levels did not increase until June. Total lipids must therefore be filling in April for the continued supply of fat to increase levels in the mesenteric store by May. The early onset of replenishment, rather than the rate of replenishment, is the significant difference which distinguishes the fat dynamics of maturing and nonmaturing fish.

Fasting during spring months (April and May), when fat stores are being replenished, had the greatest effect on fat levels. It delayed replenishment of the mesenteric store until June (Fig. 2B). The suppression of maturation rates in fasted fish groups was therefore due to a delay in replenishment of mesenteric fat, as occurred for nonmaturing fish in control groups.

Johnston et al. (1987) also concluded that the timing of fat deposition was important for maturation to proceed. They noted that oogenesis and vitellogenesis proceeded in reconditioned, previously spawned salmon (kelts), which restored fat and protein reserves early in spring. It did not occur in the fish which were slow to restore energy reserves. Unfortunately, data on

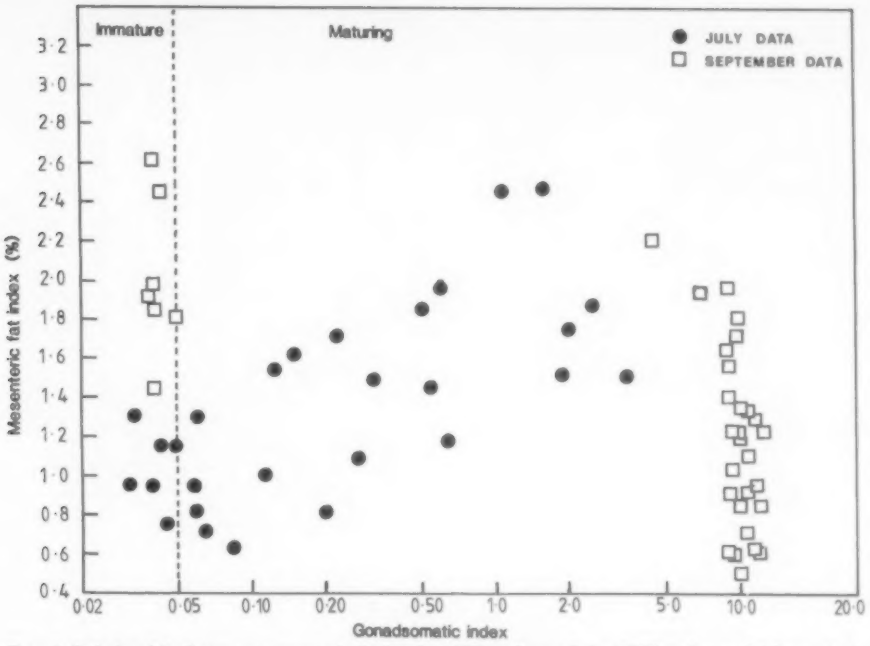

FIG. 4. Relationships between mesenteric fat levels (MFI) and gonad size (GSI on log scale) for maturing and nonmaturing male parr between July (●) and September (□) 1987.

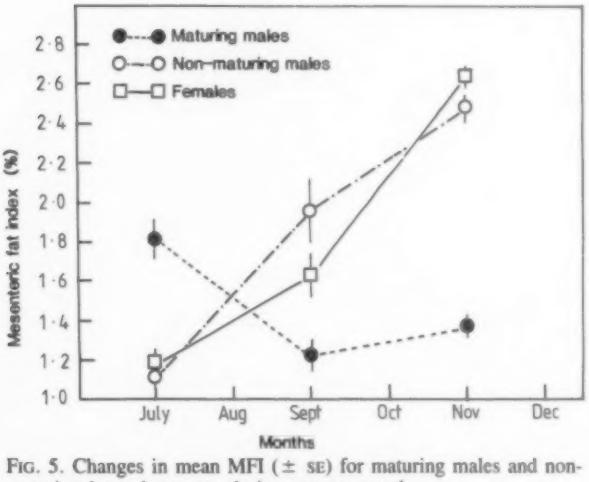

FIG. 5. Changes in mean MFI (± SE) for maturing males and nonmaturing 1+ salmon parr during autumn months.

TABLE 2. ANCOVA for log fat weight and log fish weight between food-restricted and seasonal control populations of salmon parr. $^{***}p < 0.001$.

Source of variation	df	Sum of squares	Mean square	Variance ratio
Between fasted and control populations adjusted	1	0.356	0.356	54.69***
Between months adjusted	5	0.356	0.071	10.93***
Interaction adjusted	5	0.271	0.054	8.34***
Residual adjusted	227	1.478	0.007	

feeding rates of maturing male parr were not obtained in this study during spring months, and so it is not known whether maturing males began feeding earlier than nonmaturing males. However, Johnston et al. (1987) noted that reconditioned kelts, which began feeding in January and February, rematured whereas kelts which began feeding later did not.

Role of Mesenteric Fat

Maturing male parr in control groups had significantly higher MFI's than other fish by the end of May (Fig. 2A). In the stock population this difference was still apparent by mid-July, when gonadal recrudescence had started (Fig. 5). However, the rate of increase in mesenteric fat declined in maturing fish during June (Fig. 2A), and the relative size of the mesenteric fat store in maturing male parr declined between July and September (Fig. 5).

The decline in mean MFI of maturing parr between July and September could not be accounted for by changes in the size of the fish at this time and was due to an actual reduction in size of the mesenteric store. This depletion coincided with reduced feeding relative to nonmaturing parr (Rowe and Thorpe 1990b) and with marked increases in gonad size.

Leyzerovich (1973) also recorded an autumnal decline in mesenteric fat of maturing male parr. Furthermore, the autumnal decline in total lipids of maturing versus nonmaturing parr, noted by Saunders and Sreedharan (1977) and Koch and Bergstrom (1978), may also reflect depletion in mesenteric fat levels.

The fat from the mesenteric store is therefore mobilized in maturing fish to support the energetic costs incurred by gonad differentiation and/or the elaboration of secondary sexual characteristics, such as skin thickening (Murphy 1980) and the lower jaw elongation we observed in some male parr. Shulman (1974)

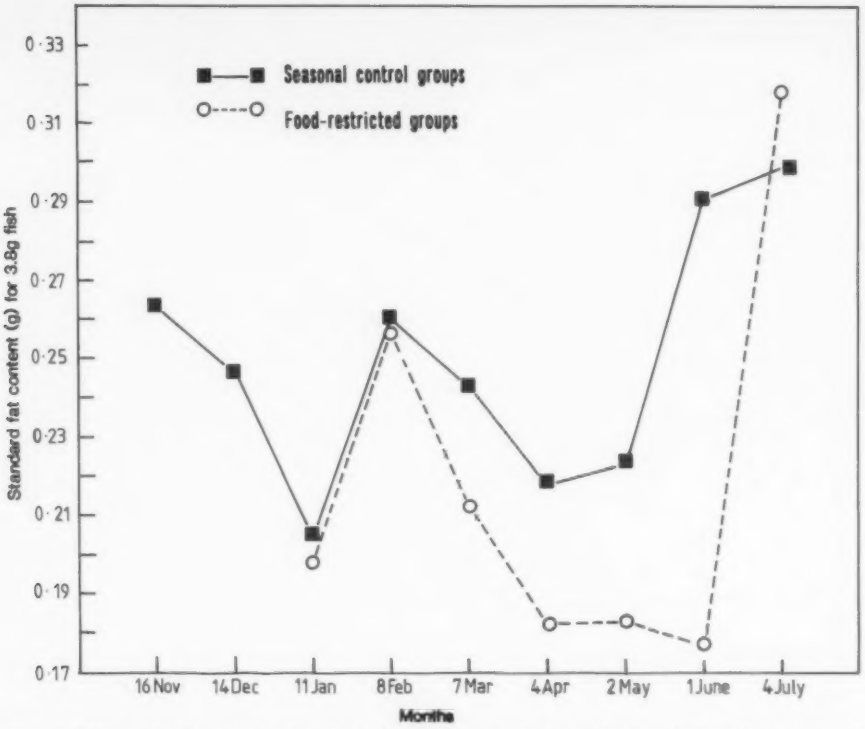

FIG. 6. Effect of food restriction (fasting during alternate weeks for an 8-wk period) on fat contents of 1+ salmon parr (values are "fitted" from regressions of fat content on weight for fasted and control groups using a common slope determined during ANCOVA).

noted that fatness declined markedly at this stage of ripening in many fish species. In Atlantic salmon parr this depletion in maturing fish occurs while nonmaturing fish are increasing fat reserves for overwintering.

Fat Dynamics and Maturation

Fat levels must be increasing during April for maturation to occur in Atlantic salmon. Early feeding is needed for this, but the fats in food must also be stored. Insulin levels peaked during April in Atlantic salmon (Dickhoff et al. 1989) and would lead to increases in fat stores, provided that water temperatures and food availability allowed growth to occur. Thus, during April, mean lipid content of maturing males increased by 46% for a 25% increase in mean weight.

Although increases in fat levels are needed during April, the decision to proceed with or to suppress maturation is made later. Fasting during May–June restricted fat deposition, mainly during May rather than June, and could not have affected fat levels in April. Nevertheless, maturation rates were suppressed (Rowe and Thorpe 1990a). The internal decision to suppress maturation is therefore left until May, despite the necessity for increases in fat during April. The decision to continue with maturation is therefore dependent on high mesenteric fat levels in May, but is sensitive to growth and increases in total fats in April, through the dynamics of fat storage.

In May the size of the mesenteric fat store is important for the decision to proceed with maturation or to suppress it whereas in autumn its contents are needed to fuel the diverse physiological changes involved in maturation. This dual role of fat stores (i.e. control of maturation and nutritive functions) also occurs in higher vertebrates.

Maturation in male and female newts (*Triturus viridescens*) was suppressed when their fat bodies were removed (Adams and Rae 1929). The fat bodies were thought to influence maturation through a nutritive role, mainly because the two tissues have a common embryological origin (Adams and Rae 1929). Fat stores are needed to fuel gonadal development, but the decision to begin gonadal recrudescence is more likely to be associated with the physiological process linking the level, or rate of increase in fat reserves, with hormonal events controlling gonadotropin (GTH) production. Removal of fat bodies in newts influenced this process.

This dual role of fat stores in the control of maturation in Atlantic salmon is likely to be adaptive. Fat stores are needed for overwintering, and mortality is higher for fish with low fat reserves (Gardiner and Geddes 1980). Rowe and Thorpe (1990a) suggested that maturation is suppressed when the probability of acquiring sufficient fat reserves to fuel gonad growth and survive the winter is low.

Physiological Model for the Control of Maturation

McCormick and Naiman (1984) produced a conceptual model for the effect of growth on the hormonal control of maturation in brook trout (*Salvelinus fontinalis*). However, while attempting to integrate the interplay of genetic factors, growth, and photoperiod in determining increased production of GTH, and hence the initiation of maturation, it is too general to be tested. It indicates that growth influences the hormonal

systems controlling maturation, not how this occurs. An alternative model linking growth in lipid stores during spring months with the hormonal control of maturation is proposed.

Frisch (1988) discussed the control of maturation and ovulation in women and hypothesized that the hypothalamic production of gonadotropin releasing hormone (GtRH) was dependent on the levels of estrogens aromatised from testosterone in fatty tissue, and hence on the amount of fatty tissue itself. Such a mechanism may be involved in the control of maturation in other vertebrates, including fish.

For example, Siiteri (1987) found extremely high testosterone aromatisation rates and estrogen levels in male squirrel monkeys (*Saimiri sciureus*) during the breeding season. Males of this species are unusual in that they increase in weight by up to 40% during the breeding season, mainly as a result of the accumulation of upper body fat.

Aromatisation of testosterones to estrogens also occurs in salmonid fish, but is believed to be restricted to the brain. Lambert and van Bohemen (1978) suggested that the brain must be the major source of estrogen in rainbow trout (*Oncorhynchus mykiss*) because preliminary results (not reported) showed that other tissues, including fats, did not contain aromatising enzymes. Lambert et al. (1982) subsequently recorded aromatase activity in various brain tissues of salmonids, and Callard (1982) indicated that such aromatisation needs to be site specific in fish, and present in the brain or pituitary, as the gills remove most circulating estrogen. Recently, Andersson et al. (1988) found aromatase activity in the brain and pituitary of Atlantic salmon parr, the highest levels occurring in extracts from mature male parr.

Although it is apparent that aromatase activity occurs in the brain and pituitary of fish, including male Atlantic salmon parr, there is little direct evidence for its absence in other tissues, particularly fat tissues. In female rainbow trout, steroidogenesis takes place in both granulosa and stromal cells of the ovary, and aromatase enzymes synthesise estrogens in the ovary from May to August, but not during previtellogenesis in March (Lambert et al. 1978). In humans, most aromatase activity is found in the stromal (interstitial) cells of adipose tissue (Cleland et al. 1983), more occurring in the omental than subcutaneous fat (Ackerman et al. 1981). Thus, aromatase activity occurs principally in stromal cells, but the extent of activity is likely to vary depending on the tissue in which the stromal cells occur, the maturation status of the fish, and the time of year. Such factors need to be considered in studies to rule out the absence of aromatase activity in fatty tissues of fish.

Such studies have not been reported yet. However, weight of circumstantial evidence suggests that Frisch's hypothesis for the control of maturation, by aromatisation of androgens in fatty tissue, applies to salmon parr.

Maximum aromatase activity is likely to occur when seasonal cortisol levels are highest. Simpson et al (1981) found that aromatase activity in humans was stimulated 20- to 100-fold by glucocorticosteroids including cortisol. Extraordinarily high levels of cortisol (50–100 times higher than in humans and old world primates) occur in the male squirrel monkey during the breeding season (Klosterman et al. 1986). Estrogen and testosterone levels in males are also high at this time, and Siiteri (1987) concluded that these high cortisol levels would account for the high plasma estrogen levels, through the aromatisation of testosterone in the extensive upper body fat stores.

In Atlantic salmon parr, highest cortisol levels occur during spring months, with a peak in March (Thorpe et al. 1987). Maximum aromatase activity could therefore be expected at this time, provided testosterone was available for conversion.

Small peaks in testosterone and 11-ketotestosterone occur in Atlantic salmon plasma during February to March (Abdullah 1981; Hunt et al. 1982; Youngson and McLay 1985), and fish with elevated androgens during this period all matured. In comparison, most fish not showing such spring peaks failed to mature.

This testosterone is likely to come from the head kidney. Yamada et al. (1988) found that the head kidney was the major source of testosterone in masu salmon (*Oncorhynchus masu*) during spring months. Production was high in April but decreased during May and June. In comparison, testosterone production by the gonad was comparatively low during April and May, and only began to increase in June.

These spring peaks in extragonadal testosterone levels of prepubescent salmonids are believed to be functional (Elliott et al. 1984) and have a role in gonadal recrudescence. For example, testosterone implants in male Atlantic salmon parr stimulated precocious development (Crim and Evans 1982), probably through the effects of testosterone on GtH production by the pituitary.

Peter (1982) and Goos (1987) both noted that positive feedback by testosterone on pituitary GTH levels is important during the prepubescent period of gonadal development. Testosterone treatment has resulted in both pituitary synthesis and release of GTH in a number of salmonids, and stimulated GtRH activity in the hypothalamus (Goos et al. 1986). These and other studies have shown that above-normal levels of testosterone influence the hypothalamus and pituitary, stimulating gonadal development via production and release of GTH by the pituitary. However, testosterone may not have a direct effect on the hypothalamus or pituitary. It may only provide the substrate for aromatisation to estrogen.

Crim et al. (1981) demonstrated that only estrogens and aromatizable testosterones stimulated GTH production in trout. More significantly, they found that an aromatase inhibitor reduced GTH production in testosterone-treated fish. Estrogen, derived from the aromatisation of testosterone, was apparently required to stimulate gonadal recrudescence in testosterone-treated fish.

Prematuration peaks in plasma estrogen levels occur in salmonids during spring months (Billard et al. 1978; Elliott et al. 1984; Sower et al. 1984; Yamada et al. 1988), and there is evidence that this estrogen is aromatised from testosterones (Sower et al. 1984; Yamada et al. 1988). The aromatisation of testosterone may be a significant source of estrogen production in female as well as male fish (Greeley et al. 1988).

The seasonal timing of prematuration peaks in cortisol, testosterones, and estrogens coincide with growth in fat reserves in maturing Atlantic salmon parr. Fat stores are linked with the control of maturation in these fish, and the coincidental timing of peaks in hormone production is consistent with Frisch's hypothesis for the influence of fat stores on the initiation of maturation. It is apparent that estrogen primes the hypothalamus and pituitary in these fish, and that aromatisation of testosterones to estrogens is involved in the control (if not initiation) of gonadal recrudescence. A major site for aromatisation activity, in addition to the brain, may be fat stores. Such a mechanism could explain how the level of mesenteric fat in May influences maturation.

Alternatively, the low levels of fat in nonmaturing fish may reflect a shortage of cholesterol, which is the precursor for sex

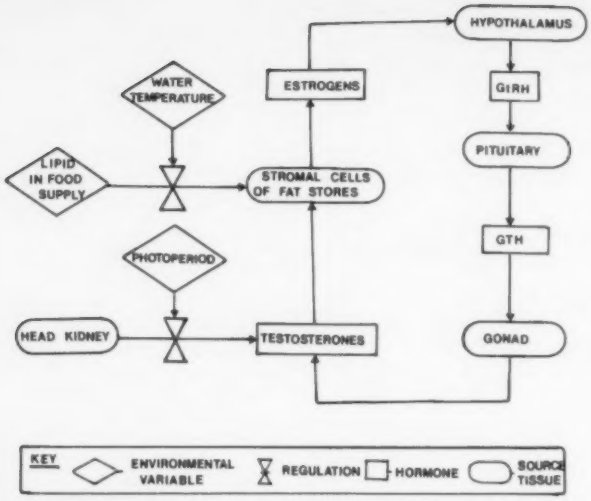

FIG. 7. Hypothetical systems model for the environmental regulation of the positive hormonal feedback loop controlling maturation in Atlantic salmon (GtRH = gonadotropin releasing hormone, GTH = gonadotropin).

hormones. Poor growth during spring may influence maturation by limiting concentrations of cholesterol available for synthesis of testosterone in the head kidney. For example, Koch and Bergstrom (1978) found that cholesterol levels of mature male Atlantic salmon increased by over 25% from 400 mg/100 mL to 550 mg/100 mL in March and April (i.e. 4 mo before gonadal recrudescence). In comparison, cholesterol levels of immature parr remained low at 350 mg/100 mL.

If cholesterol, and hence testosterone, levels were limiting then testosterone implants, prior to gonadal recrudescence, should result in all male fish maturing. Magri et al. (1985) found that testosterone implants, inserted 8 mo before the onset of gonadal recrudescence, stimulated maturation in 50% of male parr of rainbow trout. The failure of many of the implanted males to mature was unlikely to have been due to a lack of hormone substrate, at least at this time. Some other factor prevented maturation from occurring in these fish.

Rowe and Thorpe (1990a, 1990b) have indicated that growth opportunity during spring months influences maturation. In this paper we provide evidence that the size of the mesenteric fat store in May is important for maturation to proceed. We propose that fat levels exert control over maturation by limiting the extent of aromatase activity, and hence the production of estrogens needed to stimulate production of the appropriate GTH.

This physiological model for the hormonal control of maturation (Fig. 7) is based on a positive feedback loop by which estrogens are produced from the aromatisation of androgens. Dufour et al. (1989) also suggested that a positive feedback loop, stimulated by prepubescent GTH, amplifies hormone production and leads to gonad growth. In salmonids, the feedback loop would be primed by testosterones from the head kidney in late winter and regulated by the size of lipid stores during spring. The time at which the feedback loop is primed by the production of extragonadal testosterone is likely to be endogenously regulated through photoperiod. The hereditary influence on age at maturation (cf. Thorpe et al. 1983) is probably due to genetic differences in physiological factors such as testosterone production rates from the head kidney, aromatisation rates, sensitivity to estrogen levels, or factors influencing fat dynamics. Such factors are likely to account for the stock-specific differences in sensitivity to spring growth (i.e. the stock-specific thresholds of Thorpe's (1986) model).

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Adrenergic Response to Physiological Disturbances in Rainbow Trout, *Oncorhynchus mykiss*, Exposed to Aluminum at Acid pH

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A 10-fold increase of plasma epinephrine and norepinephrine levels was evident at 46 h of Al exposure in adult rainbow trout, *Oncorhynchus mykiss*, which were kept for about 2.5 d at pH 5.0 with 60 μg Al/L (Ca^{2+} = 28 μmol/L). The change of plasma epinephrine levels was related both to the decrease of the blood pH and the decrease of the blood Po_2. We further observed decreased plasma Na^+ concentrations which were accompanied by elevated levels of cortisol in the plasma of Al-exposed fish. Exposure of fish to pH 6.8 (=control) or pH 5.0 without Al did not yield any changes in plasma Na^+ concentrations, plasma cortisol concentrations, blood pH, blood Po_2, and plasma epinephrine, norepinephrine, and dopamine levels. The release of plasma catecholamines associated with blood acidosis and hypoxia is suggested to be an important factor in maintaining erythrocytic pH to protect the haemoglobin oxygen carrying capacity in fish exposed to low pH and Al.

La multiplication par 10 des concentrations de norépinéphrine et d'épinéphrine plasmatiques était évidente après 46 h d'exposition à l'Al des truites arc-en-ciel adultes, *Oncorhynchus mykiss*, tenues pendant environ 2,5 jours à un pH de 5,0 avec 60 μg Al/L (Ca^{2+} = 28 μmol/L). Le changement observé dans les concentrations d'épinéphrine du plasma était en rapport avec la baisse du pH et du Po_2 sanguins. Nous avons en outre observé une réduction des concentrations de Na^+ du plasma, accompagnées de concentrations élevées de cortisol dans le plasma des poissons exposés à l'Al. L'exposition des poissons à pH 6,8 (= groupe témoin) ou à pH 5,0 dans un milieu exempt d'Al ne s'est accompagnée d'aucun changement dans les concentrations de Na^+ et de cortisol plasmatiques, dans le pH et le Po_2 sanguins, et dans les concentrations de dopamine, de norépinéphrine et d'épinéphrine plasmatiques. On pense que le dégagement de catécholamines plasmatiques, associé à l'hypoxia et à l'acidose sanguines, pourrait être un important facteur dans le maintien du pH des érythrocytes pour protéger la capacité de transport de l'oxygène de l'hémoglobine dans le poisson exposé à de faibles concentrations de pH et d'Al.

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Recent evidence suggests that Al, leached from acid-sensitive soils (Johnson et al. 1981), contributes to the high mortality of freshwater organisms in acidified lakes and streams (for reviews see Witters and Vanderborght 1987; Schindler 1988).

Low ambient pH causes severe disturbance of ion balance in fish (Neville 1979; McDonald and Wood 1981; McDonald 1983) which is exacerbated by the presence of Al (Witters 1986; Booth et al. 1988; Wood et al. 1988a; Playle et al. 1989). In addition, respiratory failure (mucus production with concomitant decrease of O_2 and CO_2 permeability) has been demonstrated in fish under Al stress at pH levels which have little effect on gas exchange (Neville 1985; Walker et al. 1988; Wood et al. 1988a). High haematocrit levels (40–60%) have been observed in fish exposed to Al and low pH (Witters 1986; Goss and Wood 1988; Witters et al. 1987; Booth et al. 1988; Wood et al. 1988a). Previous studies (Witters et al. 1987, 1990) showed that both an increased mobilization of erythrocytes from the spleen into the blood and an increased swelling of erythrocytes contributed to the high haematocrit values. These haematological changes may be initiated by a release of catecholamines in the plasma of Al-exposed fish. Nilsson and Grove (1974) remarked that arterial injection of catecholamines in the cod, *Gadus morhua*, induced contractions of the spleen. Such splenic contractions and a release of red blood cells from the spleen were observed by Yamamoto et al. (1985) in fish under hypoxic conditions during exercise. Nikinmaa (1982, 1983, 1986) showed that plasma catecholamines could induce cell swelling to maintain intracellular pH. This mechanism may optimize oxygen transport during hypoxic conditions.

Data on plasma catecholamine levels in fish exposed to acid or acid with Al have not been reported previously. In this study, adult rainbow trout were kept for 3 d in water at pH 5.0 or at pH 5.0 with 60 μg Al/L. Differences in blood pH and blood Po_2 were measured and compared with measured plasma catecholamine levels. The levels of cortisol and Na in the plasma were followed as indices of ion balance disturbance. Cortisol can also be mobilized during stress (Brown et al. 1984; Goss

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TABLE 1. pH and ion composition (μmol/L) of the acclimation water and the experimental solutions (mean values ± 95% confidence limits).

	Acclimation control	Acid	Acid + Al
pH	6.8 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
Na^+	457 ± 26	456 ± 15	422 ± 12
Ca^{2+}	28 ± 2	27 ± 1	27 ± 1
Cl^-	346 ± 17	339 ± 20	387 ± 20
Al_{tot}	—[a]	—[a]	2.3 ± 0.2 (60 ± 5 μg/L)

[a]Below detection limit of Al (<1.1 μmol/L).

and Wood 1988) and may have a role inducing chloride cell proliferation (Doyle and Epstein 1972).

Materials and Methods

Experimental Animals and Holding Conditions

Adult rainbow trout of both sexes (weight range 180–320 g) were purchased from a hatchery in France (Bretagne). They were held indoors for at least 2–3 wk in 1000-L fiberglass tanks supplied with aerated, flowing tap water (pH 8.6; $[Na^+]$ = 0.4; $[Ca^{2+}]$ = 1.0; $[Cl^-]$ = 0.3; $[HCO_3^- + CO_3^{2-}]$ = 0.9 mmol/L, temperature = 16°C). Fish were fed regularly with trout pellets until 10 d prior to experimentation. To acclimate fish to the experimental conditions (temperature = 14°C), they were moved to and kept in darkened tanks (150 L) for 8 d prior to beginning the experiments. Water of low ionic content was continuously supplied (60 L/h) to each tank.

The acclimation water (= the same as control water in the experiments) was prepared by addition of mineral salts (NaCl, KCl, $CaCl_2$, $MgSO_4$, and $NaHCO_3$) with dosing pumps (Prominent Electronic, E1201) to the flowing demineralised water (Table 1), which was obtained by ion exchange. The acclimation water of pH 6.8 (Table 1) passed into a 100-L reservoir and was continuously acidified to pH 5 before it left the reservoir to enter the experimental tanks. Acidification was achieved through addition of an acid solution prepared as a mixture of 2 volumes of H_2SO_4 and 1 volume of HNO_3 (0.1 N). This was added with another dosing pump to establish the pH of the experimental solutions in the 100-L reservoir using an automatic controlling device (Prominent Dulcometer PHWSO14) connected to the acid-dosing pump. The Al was added to the acid water in the 100-L reservoir from a stock solution (100 mg Al/L as $AlCl_3$) using dosing pumps.

The pH in the fish tanks was checked for a second time, since it could change due to excretion of the fish, and it was adjusted appropriately when necessary by another automatic device constructed by our technical department (SCK/CEN). The water in the fish tanks was continuously aerated and was recirculated at a rate of 1000 L/h. During the experiments, fish remained in individual fish chambers, which were supplied with water from the fish tanks by recirculation (1000 L/h) (Eheim, type 1036).

The water pH was regularly controlled with a Corning electrode (general purpose, type 476531), coupled to an Orion research pH meter (model 701A). The ion concentration in the experimental solutions (Table 1) was measured either by plasma emission spectrophotometry (Jarrel Ash, Atomcomp, model 750) for the cations (Na, Ca, Mg, K, Al_{tot}) or by segmental flow analysis (Skalar, model 5100) using a colorimetric technique for Cl (Greenberg et al. 1980).

The chemical speciation of Al in the experimental solutions was analysed by the method of Driscoll (1984). About 95–100% was found to occur as inorganic monomeric Al.

Experimental Procedure

Arterial catheters were implanted in fish via the dorsal aorta about 48 h before the start of the experiments. Fish were anaesthesized in a solution of 100 mg tricaine methanesulfonate/L (Sigma) in the acclimation water. Surgery was performed as described by Witters et al. (1990). After the operation, fish were placed in Plexiglas tubes to restrict movement. The tubes were placed in individual chambers (11 cm wide × 40 cm long × 30 cm high), which were covered at the outer sites with a black plastic film. Each fish chamber was individually aerated. A preliminary blood sample (t = 0) was taken as a reference value. Then the water composition was changed for the experimental treatments. The acclimation water was partly removed in two fish tanks (±30%) and experimental water (acid or acid with Al) was added at a rate of 60 L/h and allowed to mix. The pH value (pH 5.0) and the Al concentration (60 μg Al/L) were reached within 3–4 h in either acid or acid with Al exposure series. In the control treatment (third fish tank), fish were kept in the acclimation water over the whole experimental period.

Further blood samples were taken at 4, 22, 28, 46, and 52 h after pH and Al content had established in the fish tanks. Daily blood samples were taken at the same hours (9:30 a.m. and 3:30 p.m.) to limit the effect of diurnal rhythm on plasma hormone levels. Blood samples of 450 μL were removed with a 500-μL gas-tight syringe (Hamilton), and an equivalent amount of heparinized (Li-heparin, Fluka) Ringer solution (Olson 1984) was injected to replace lost blood. About 40 μL of the sampled blood was immediately injected in the capillary of a blood gas analyzer (AVL, Gascheck type 939). About 400 μL was placed in ice-cooled tubes which contained 10 μL of a solution of 90 mg EGTA/mL (anticoagulant) (Merck, pro analysis) and 60 mg reduced glutathione/mL (to prevent oxidation of catecholamines) (Merck). An ammonium heparinized glass capillary (Clay Adams) was filled with blood for haematocrit determination by centrifugation (IEC, model MB). The remainder was centrifuged (Heraeus, Biofuge A) for 3 min at 11 000 × g. Pasma was separated off and 25 μL was diluted with 0.01 N HCl (1:200) for plasma Na concentration measurements. The rest of the plasma was frozen in liquid nitrogen (−70°C) for determination of cortisol and catecholamine concentrations within 2–3 wk.

Analysis of Blood and Plasma Variables

The Na concentration in the diluted plasma samples was determined by plasma emission spectrophotometry (Jarrell Ash, Atomcomp, model 750). The cortisol levels were determined in the thawed plasma samples with a commercially available RIA technique (Amersham, Amerlex Cortisol Ria kit, code IM 2021). Plasma was assayed for epinephrine, norepinephrine, and dopamine levels by a radioenzymatic assay method with a commercially available kit (Amersham, Cat-A-kit, code TRK 895). Thin-layer chromatography on silica gel plates (Kieselgel, type 60F254) in a solvent of tertiary amyl alcohol – benzene – methylamine (6:2:3) was used to separate the catecholamine derivates. Catecholamine standards were determined with the analyses of plasma samples to ensure accuracy of the obtained

values. Catecholamine levels were only determined in plasma samples (2×50 μL) taken during the preexposure period at $t = 0$ and on samples taken after 46 h of exposure to water of pH 6.8 (=control), pH 5.0, and pH 5.0+60 μg Al/L. Catecholamines were not analyzed at each sampling time because of the high cost of the kit and the time-consuming analyses. Only catecholamine levels measured in fish that survived the 52-h exposure were included in the results.

Statistical Analysis

The values presented in the tables and in the figures are mean values with 95% confidence limits. Statistical differences within groups (control, acid, or acid + Al) were evaluated by a two-tailed Student *t*-test with the appropriate degrees of freedom in comparison with the reference value at $t = 0$ for each of the experimental values at different exposure times (Fig. 1). Comparison between groups was performed by a two-level ANOVA (mixed model) for unequal sample sizes (Sokal and Rohlf 1969). A modification of the Student *t*-test, Cochran's approximation (Snedecor 1957), was applied for comparison of catecholamine levels between groups because of heterogenity of variances (*F*-test, Sokal and Rohlf 1969). The chosen level of significance is $P < 0.05$. A least squares linear regression programme which computes the slope, the *y*-intercept, and the correlation coefficient *r* was used (Sokal and Rohlf 1969).

Results

Mortality

Only one fish died in both the control group ($n = 20$) and in the acid exposure group ($n = 24$). Mortality was significantly higher in fish ($n = 34$) exposed to pH 5.0 with 60 μg Al/L, being 40% within 52 h of exposure.

Physiological Responses

The control exposure (pH 6.8) and acid exposure (pH 5.0) had no effect on plasma Na concentration (Fig. 1A), plasma cortisol concentration (Fig. 1B), blood Po_2 (Fig. 1C), and blood pH (Fig. 1D). The significant decrease of the haematocrit value (Fig. 1E), both at pH 6.8 and at pH 5.0, can be explained as an effect of repetitive blood sampling. Data on the levels of epinephrine, norepinephrine, and dopamine (Table 2) illustrate that fish exposed to pH 6.8 or pH 5.0 did not experience any physiological stress at the times examined.

The exposure of fish to pH 5.0 with 60 μg Al/L, on the other hand, induced significant disturbances of the measured physiological parameters (Fig. 1; Table 2). In spite of repetitive blood sampling, a significant increase of the haematocrit value was observed in the presence of 60 μg Al/L (Fig. 1E). Concomitant with the decreases of the plasma Na concentration (Fig. 1A), significantly higher plasma cortisol levels (Fig. 1B) were observed. A severe blood acidosis (Fig. 1D) also occurred in Al-exposed fish. Because no other parameters of the acid–base balance were measured, it could not be determined whether the blood acidosis was of metabolic or respiratory origin or a combination of both. The decrease of blood Po_2 values provided evidence for respiratory problems in Al-exposed fish (Fig. 1C). Comparison among experimental groups (acid and acid with Al) (ANOVA) revealed significant ($P < 0.05$) added variance for plasma cortisol, blood Po_2, blood pH, and haematocrit, which can be attributed to the presence of Al in acid water. This additive stress of Al on fish is supported by the Al-induced increase of epinephrine and norepinephrine levels in fish plasma after 46 h of exposure. Mean plasma epinephrine levels are 13 times higher and mean norepinephrine levels are 7 times higher in Al-exposed fish compared with preexposure values, but Al had no influence on plasma dopamine levels (Table 2).

For the limited number of fish data on blood pH, blood Po_2, and plasma epinephrine and norepinephrine that were available from both sampling times $t = 0$ (control) and $t = 46$ h of exposure (acid or acid with Al), we found that the increase of plasma epinephrine levels was significantly related to the change of blood Po_2 as well as to the blood pH (Fig. 2).

Discussion

Acid exposure to pH 5.0 for 52 h caused no significant changes of the measured physiological parameters in rainbow trout. These results are consistent with the findings of Neville (1985) which demonstrated that neither the plasma NaCl balance nor respiratory parameters such as ventilation rate and oxygen consumption were influenced during 6–11 d of exposure of rainbow trout to pH 5.0.

Mortality data in this study indicated the highly toxic action of low levels of Al (60 μg Al/L) at pH 5.0. In our experimental setup which makes use of artificially prepared water, Al is likely to occur as the aluminium ion Al^{3+} and aluminium hydroxide forms $Al(OH)_2^+$ and $Al(OH)^{2+}$ at pH 5.0. Playle and Wood (1989) showed that Al deposition occurs at the gills due to increased pH in the branchial microenvironment above that of the inspired acid water. Higher pH values may promote Al precipitation and Al accumulation (Witters et al. 1987; Booth et al. 1988; Goossenaerts et al. 1988; Neville and Campbell 1988) due to the formation of hydroxide forms with lower solubility (Playle and Wood 1989). In this way, Al could act as an irritant to the gill surface, stimulating mucus production and decreasing branchial permeability for CO_2 and O_2 exchange (Malte and Weber 1988; Walker et al. 1988; Wood et al. 1988a). Moreover, distortion of the gill epithelium may contribute to greater permeability of paracellular channels and subsequent elevated loss of electrolytes (Witters et al. 1987; Booth et al. 1988; Wood et al. 1988a; Wood 1989). The exacerbated decrease of plasma Na levels in fish exposed to Al (Fig. 1A) agrees with our previous findings (Witters 1986; Witters et al. 1987) and studies by Goss and Wood (1988), Booth et al. (1988), and Wood et al. (1988a). Elevated cortisol levels (Fig. 1B) in Al-stressed rainbow trout seem to be a sensitive indicator of stress, as suggested by Goss and Wood (1988). Cortisol levels in resting conditions were between 150 and 250 nmol/L. Cortisol measurements in control fish (Fig. 1B) showed little evidence of a diurnal rhythm (Bry 1982; Pickering and Pottinger 1983) which was probably eliminated by the permanent dark exposure and the starvation of the fish (Pickering and Pottinger 1983). No stress effects of regular blood sampling on plasma cortisol levels in cannulated rainbow trout were observed (Fig. 1B). Thus the high cortisol levels reported in Fig. 1B were presumably an immediate response to Al stress. The mobilization of cortisol from the interrenal tissue (Brown et al. 1984) under acidic conditions could have two effects. First, it may mediate glycogenolysis, resulting in hyperglycemia which might help to maintain plasma osmolality and to provide energy for ionoregulatory mechanisms. Elevated levels of glucose in Al-stressed fish were measured by Goss and Wood (1988) and

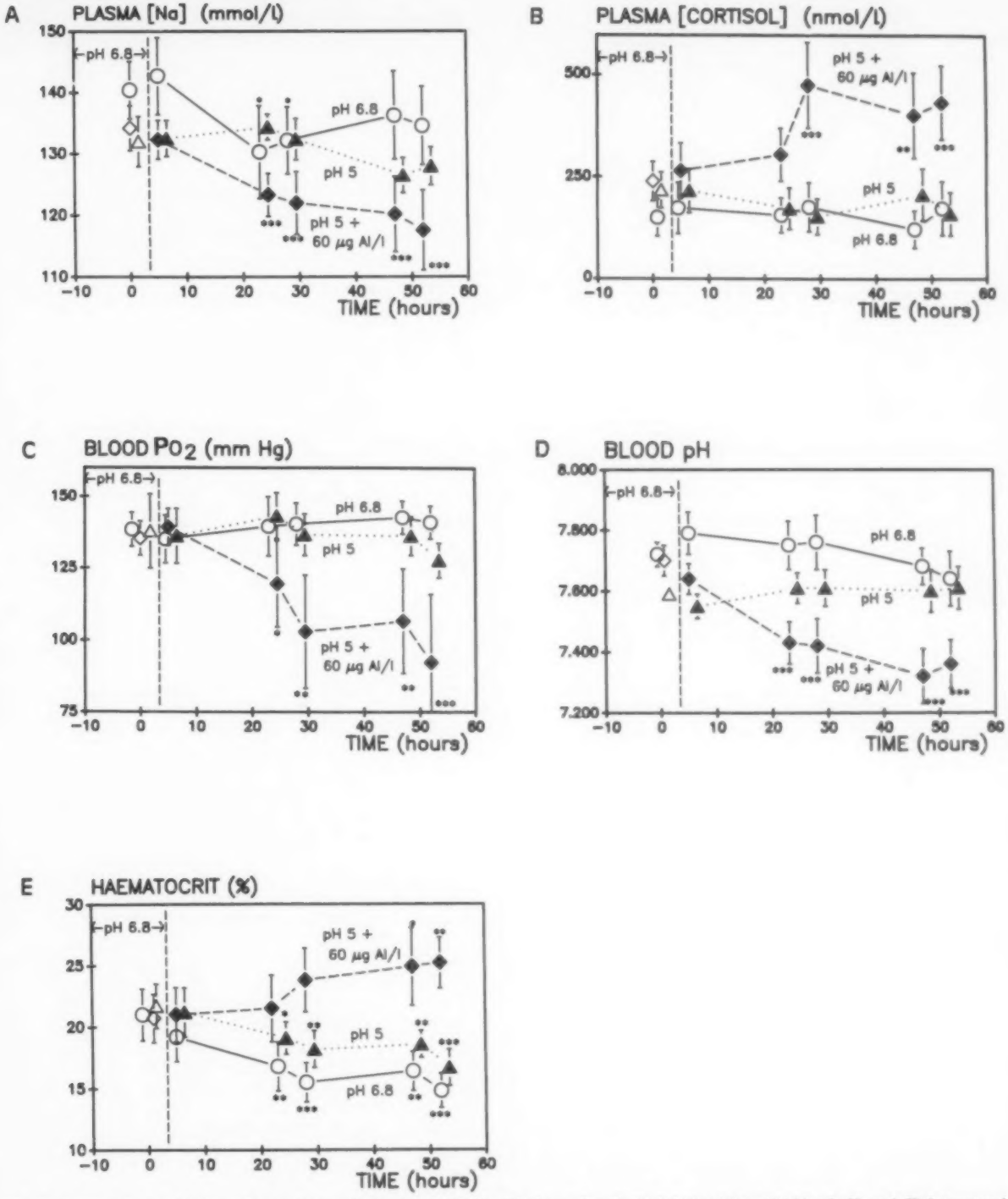

FIG. 1. Mean values ± 95% confidence limits of (A) plasma [Na^+], (B) plasma [cortisol], (C) blood Po_2, (D) blood pH, and (E) haematocrit in cannulated rainbow trout exposed for 52 h to pH 6.8, pH 5.0, or pH 5.0 with 60 µg Al/L. Asterisks indicate significant differences with the corresponding preexposure values at $t = 0$, calculated with a two-tailed Student t-test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Witters et al. (1990). Second, cortisol could aid with ionoregulation by stimulating chloride cell proliferation at the branchial epithelium. Chloride cells are thought to represent the sites for ion exchange in fish gills, and cortisol treatment increased their numbers in the eel, *Anguilla rostrata* (Doyle and Epstein 1972), and in rainbow trout (Perry and Wood 1985).

TABLE 2. Concentration of plasma catecholamines (nmol/L) in cannulated rainbow trout (n = number of sampled fish) under different experimental conditions. Mean values ± 95% confidence limits are given. Significant differences were tested with a modified Student t-test, the Cochran's approximation, and are indicated by asterisks: $^*P < 0.05$.

	Preexposure (t = 0)	Experimental treatment (t = 46 h)		
	pH 6.8 (n = 18)	pH 6.8 (n = 5)	pH 5.0 (n = 11)	pH 5.0 ± 60 µg Al/L (n = 17)
Epinephrine	1.00 ± 0.43	0.49 ± 0.58	1.10 ± 0.56	13.8 ± 9.8
			*	*
		*		
		*		
Norepinephrine	1.94 ± 0.59	1.19 ± 1.67	1.85 ± 0.63	12.67 ± 7.97
			*	*
		*		
		*		
Dopamine	2.63 ± 1.64	1.11 ± 1.09	3.38 ± 2.32	2.52 ± 1.48

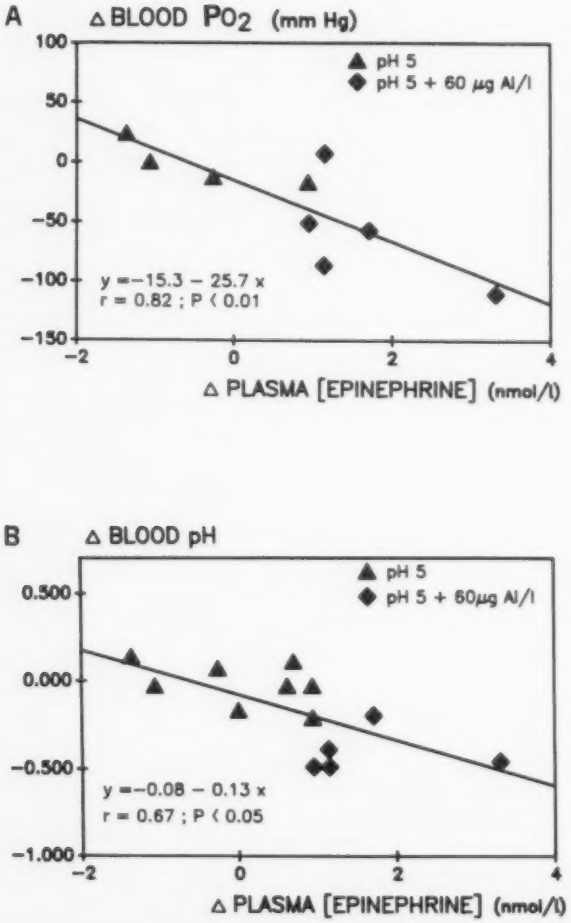
A
Δ BLOOD PO2 (mm Hg)
pH 5
pH 5 + 60 µg Al/l
y = −15.3 − 25.7 x
r = 0.82 ; P < 0.01
Δ PLASMA [EPINEPHRINE] (nmol/l)
B
Δ BLOOD pH
pH 5
pH 5 + 60µg Al/l
y = −0.08 − 0.13 x
r = 0.67 ; P < 0.05
Δ PLASMA [EPINEPHRINE] (nmol/l)

FIG. 2. Relationship showing changes of (A) blood P_{O_2} and (B) blood pH as a function of changes of plasma [epinephrine] between preexposure values at $t = 0$ and after 46 h at pH 5 or at pH 5.0 + 60 µg/Al. Regression was obtained by least squares fit.

Besides these osmoregulatory changes, the haematocrit levels increased (Fig. 1E) and blood P_{O_2} (Fig. 1C) and blood pH (Fig. 1D) decreased in rainbow trout at pH 5.0 with 60 µg Al/L. These results corroborate other work on Al-exposed trout (Walker et al. 1988; Wood et al. 1988a; Playle et al. 1989).

Our measurements of the plasma catecholamine levels give new information on the magnitude of the adrenergic response to changes of some physiological parameters during Al stress. The levels of catecholamines measured at $t = 0$ under control conditions correspond with previous data (Woodward 1982; Ristori and Laurent 1985; Tang and Boutilier 1988; Gingerich and Drottar 1989). Detrimental effects of surgery and regular blood sampling on catecholamine levels were not evident because the values at $t = 0$ and $t = 46$ h of exposure were similar (Table 2). Gingerich and Drottar (1989) showed that plasma catecholamines returned to baseline levels within 48 h after surgery (dorsal aorta catheterisation). Also, Ristori and Laurent (1985) and Boutilier et al. (1986) demonstrated that blood sampling had little effect. Thus the significant increase of epinephrine and norepinephrine levels (Table 2) represented a specific response to the presence of Al (60 µg/L) in the low-pH water. Epinephrine was the dominant catecholamine released during Al stress, since the ratio of epinephrine to norepinephrine increased from 0.5 ($t = 0$) to 1.1 after 46 h of Al exposure.

Catecholamine release in fish is sensitive to a variety of stress conditions (for a review see Mazeaud and Mazeaud 1981). However, knowledge of the factors controlling the discharge of catecholamines is poor. A common feature of stress in fish, related to an increased mobilization of catecholamines, is extracellular acidosis (Ristori and Laurent 1985; Boutilier et al. 1986; Primmett et al. 1986; Boutilier et al. 1988; Tang and Boutilier 1988; Vermette and Perry 1988). Tang and Boutilier (1988) gave clear evidence that the relese of catecholamines in the plasma was proportional to the degree of acidotic stress in trout.

Data in Fig. 2 indicate that the change of epinephrine levels in Al-exposed fish was related to the decrease of blood P_{O_2} and the decrease of blood pH. As outlined by Nikinmaa (1986) and Vermette and Perry (1988) the adrenergic response can include at least three factors: (1) stabilization of erythrocytic pH, (2)

an increase in P_{O_2}, and (3) an increase in blood oxygen-carrying capacity. We suggest that the role of plasma catecholamines in Al-exposed fish is to optimize both O_2 transfer and O_2 transport with reference to the haemoglobin carrying capacity during acidotic and hypoxic conditions.

Despite the extracellular blood acidosis (Fig. 1D) in Al-stressed fish, Wood et al. (1988b) found that intracellular pH of erythrocytes was unaltered. The mechanism responsible for an adrenergic control of erythrocytic pH is stimulation of the Na^+/H^+ exchanger which extrudes H^+ out of the cell against an electrochemical gradient (Nikinmaa 1986).

Increased haematocrit values in fish exposed to low pH and/or low pH with Al could be caused by (1) an increase in the number of erythrocytes due to catecholamine-induced mobilization from the spleen (Yamamoto et al. 1985), (2) swelling of erythrocytes due to osmotic shifts (Witters et al. 1987, 1990) and/or adrenergic stimulation of the Na^+/H^+ exchange (Nikinmaa 1986), and (3) a decreased plasma volume caused by fluid shifts (Milligan and Wood 1982; Witters et al. 1990). Previous experiments indicate that the haematocrit increase in Al-exposed fish could be attributed to these three factors (Witters et al. 1990). The increase of the number of erythrocytes was accompanied by an increase of the blood haemoglobin content (Witters et al. 1987), being important to optimize O_2 transport. The increase of red cell volume (Witters et al. 1987) is favourable for an increase of oxygen affinity and maintenance of intracellular pH (Nikinmaa 1982, 1986).

Thus, data from this study suggest that adrenergic responses may be important factors involved with physiological adaptation in Al-exposed fish. Haematological changes and osmotic changes resulting in increased values of haematocrit all imply an adrenergic mediated response which increases blood oxygen-carrying capacity in Al-exposed fish.

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Lack of Biological Activities in the Extracellular Products of *Renibacterium salmoninarum*

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Bandin, I., Y. Santos, D. W. Bruno, R. S. Raynard, A. E. Toranzo, and J. L. Barja. 1991. Lack of biological activities in the extracellular products of *Renibacterium salmoninarum*. Can. J. Fish. Aquat. Sci. 48: 421–425.

Evaluation in vivo and in vitro of the extracellular products (ECP) of a group of *Renibacterium salmoninarum* strains from different geographic origins revealed a low production of extracellular proteins in all strains and confirmed that most of the isolates were poor producers of proteolytic enzymes. The ECP displayed neither haemolytic activity nor capacity to alter the osmotic equilibrium of fish erythrocytes. In addition, none of the ECP samples displayed cytotoxic activities regardless of the origin of the cell line employed and did not contain substances lethal for fish, since no mortalities were recorded when doses ranging from 10 to 20 μg protein/g fish were administered. Although the present work failed to demonstrate toxicity in vivo of the ECP, it is possible that some extracellular enzymes contribute to the weakening of fish during the disease process and allow bacterial survival.

L'évaluation in vivo et in vitro des produits extracellulaires d'un groupe de souches de *Renibacterium salmoninarum* en provenance de diverses régions a révélé une faible production de protéines extracellulaires chez toutes les souches et a confirmé que la plupart des isolats étaient de faibles producteurs d'enzymes protéolytiques. Les produits extracellulaires ne montraient pas d'activité hémolytique ni de capacité de modifier l'équilibre osmotique des érythrocytes des poissons. En outre, aucun des échantillons de produits extracellulaires ne montrait d'activité cytotoxique, peu importe l'origine de la lignée cellulaire employée, et aucun ne renfermait de substance létale pour les poissons, car on n'a pas enregistré de mortalités à l'administration de doses de 10 à 20 μg de protéines/g de poisson. Bien que l'étude actuelle n'ait pu démontrer la toxicité in vivo des produits extracellulaires, il est possible que certaines enzymes extracellulaires contribuent à l'affaiblissement des poissons au cours de la maladie et permettent la survie des bactéries.

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Bacterial kidney disease (BKD) is a severe infection affecting wild and hatchery-reared salmonids throughout the world (Fryer and Sanders 1981; Klontz 1983). Despite many years of research, the pathogenesis of BKD remains poorly understood.

Several cell surface properties of the causative agent of BKD, *Renibacterium salmoninarum*, have been associated with adhesion to host cells and postulated as virulence factors. These include the capacity to agglutinate red blood cells, an autoagglutinating phenotype, and a hydrophobic cell wall (Daly and Stevenson 1987; Bruno 1988). The hydrophobic character may also contribute to intracellular survival (Young and Chapman 1978), attachment to surfaces in the environment (Austin and Rayment 1985), and invasion of salmonid ova and host endothelial cells (Evelyn et al. 1984; Bruno 1986; Bruno and Munro 1986a). However, we have observed (Bandin et al. 1989) that although the cell envelope of *R. salmoninarum* is highly hydrophobic, this organism does not actively attach to fish cell lines.

Although previous studies indicate that several *R. salmoninarum* strains produce DNAses, proteases, and haemolysins, no role has been attributed to these enzymes in vivo (Bruno and Munro 1986b). However, some bacterial toxins may cause red cell membrane damage accounting for the decrease in haematocrit and the splenomegaly observed in salmonids infected with BKD (Sizumoto et al. 1977; Aldrin et al. 1978; Kimura 1978; Bruno and Munro 1986c). In addition, Shieh (1988) has demonstrated that an undetermined exotoxin produced by the type strain of *R. salmoninarum* is responsible for fish mortalities, although the lethality was only achieved when an extremely high amount of protein was administered. Therefore, until now, neither the number nor the nature of toxic factors in the extracellular products produced by the bacterium has been determined.

In the present work we have tested the toxicity for rainbow trout (*Oncorhynchus mykiss*) and the proteolytic, haemolytic,

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TABLE 1. Origin of the *R. salmoninarum* strains used in this study.

Isolate No.	Origin	Source[a]
ATCC 33209	*Oncorhynchus tshawytscha* (USA)	ATCC
416	*Salmo salar* (Scotland)	D. W. Bruno
417	*Salmo salar* (Scotland)	D. W. Bruno
420	*Salmo salar* (Scotland)	D. W. Bruno
421	*Salmo salar* (Scotland)	D. W. Bruno
422	*Salmo salar* (Scotland)	D. W. Bruno
426	*Salmo salar* (Scotland)	D. W. Bruno
444	*Salmo salar* (Scotland)	D. W. Bruno
466	*Oncorhynchus kisutch* (Canada)	J. G. Daly
R1	Salmonid fish (Canada)	R. Lallier

[a]ATCC, American Type Culture Collection, Rockville, MD, USA; D. W Bruno, DAFS, Marine Laboratory, Aberdeen AB9 8DB, Scotland; J. G. Daly, Department of Microbiology, College of Biological Science, University of Geulph, Guelph, Ont. N1G 2W1, Canada; R. Lallier, Faculté de Médecine Vétérinaire, Université de Montréal, C.P. 5000, St-Hyacinthe, Qué., Canada.

and cytotoxic activities of the extracellular products (ECP) from a group of *R. salmoninarum* strains with different geographic origins. In addition, the efect of the ECP on the ability of red blood cells to maintain the intracellular concentration of Na^+ and K^+ was also evaluated.

Materials and Methods

Bacterial Strains

Ten strains of *R. salmoninarum* isolated from naturally occurring BKD in distinct species of salmonids from Scotland, the United States, and Canada were used in the present study (Table 1). *Renibacterium salmoninarum* ATCC 33209 was included as a reference strain. The working cultures were maintained on Mueller-Hinton agar (Difco) supplemented with 1% L-cysteine hydrochloride (Sigma) (MHA-C) and were stored frozen at −70°C in Mueller-Hinton broth (MHB-C) with 15% (v/v) glycerol. All the tests were performed with bacterial cultures grown at 15 and 20°C.

Preparation of Extracellular Products (ECP)

The ECP of *R. salmoninarum* strains were obtained using the cellophane plate technique (Liu 1957). Briefly, sterile sheets of cellophane were placed on the surface of MHA-C plates and inoculated by spreading 0.5 mL of a 7-d broth culture of each strain. After incubation for 10 d, cells were washed off the cellophane with a minimum volume of phosphate-buffered saline (PBS, pH 7.3). These cell suspensions were centrifuged at 10 000 × *g* for 30 min at 4°C and the supernatant filtered through a 0.45-μm membrane and stored at −30°C or lyophilized until needed. The protein concentration of each ECP was estimated by the method of Bradford (1976).

Enzymatic Assays

The general enzymatic activity of *R. salmoninarum* ECP and live cells was evaluated using the API-ZYM system (bioMérieux) at 15 and 20°C. The incubation period was 6 and 24 h for the crude extracts and live cells, respectively. To determine specific activities in the ECP, proteolytic and haemolytic assays were conducted.

Protease assays

Azocoll was employed for the detection of the total proteolytic activity. The assay was performed following manufacturer's instructions (Sigma). One unit of protease activity produced an absorbance reading of 1.0 at 520 nm after a 30-min assay at 37°C.

Caseinase activity was determined by a radial diffusion method using 1% agar containing bovine casein. Drops (10 μL) of each ECP were placed on the plates and incubated for 36 h at room temperature.

Gelatinase activity was assayed by a similar method using 1% (w/v) agarose gels containing 3% (w/v) gelatin (Oxoid) in PBS. Plates were flooded with 15% (w/v) mercuric chloride in 20% (v/v) HCl to enhance zones of clearing.

One unit of caseinase or gelatinase activity was defined as that which produced a zone of clearing equal in area to that produced by 1 μg of trypsin (16 and 13 mm, respectively).

Haemolytic activity

Blood from Atlantic salmon (*Salmo salar*) and rainbow trout was collected in Alsever solution. Sheep erythrocytes were supplied by Oxoid. Red blood cells were washed three times in PBS and resuspended to adequate concentration in this solution.

Haemolytic activity was evaluated by a radial diffusion method using MHA-C medium with 5% sheep, trout, and salmon erythrocytes added. Ten microlitres of each ECP was placed on the plates and incubated for 24 h at 15 and 20°C. One unit of haemolytic activity was defined as that which produced a zone of clearing equal in area to that produced by 0.1% SDS.

Haemolytic titer was determined as described by Hastings and Ellis (1985). Briefly, ECP of *R. salmoninarum* strains were diluted in twofold steps in PBS. To 100 μL of each dilution was addd 100 μL of a 1% red blood cell suspension and the mixture was incubated overnight. One haemolytic unit was expressed as the reciprocal of the highest dilution of crude ECP producing complete haemolysis.

Toxic Activities of the ECP in vivo and in vitro

Groups of 5–10 fingerling rainbow trout (3–4 g) were injected intraperitoneally with 0.15 mL of each preparation of ECP. Fish inoculated with 0.15 mL of PBS were used as controls. Fish were maintained at 15–20°C with aeration and observed daily during a period of 4 wk.

The ECP preparations were assayed for cytotoxicity as previously described (Toranzo et al. 1983) in the following fish cell lines: CHSE-214 (chinook salmon embryo), RTG-2 (rainbow trout gonad), FHM (fathead minnow peduncle), EPC (epithelioma papillosum of carp), BF-2 (peduncle of bluegill fry), and BB (caudal peduncle of brown bullhead). In addition, two homoiothermic cell lines were used: L-929 (mouse lung fibroblast) and HeLa (human cervix epithelioid carcinoma). Cell monolayers grown in 24-well plates (Costar, Cambridge, MA) were inoculated with 0.1 mL of each sample and incubated at 18°C (fish cell lines) or 37°C (homoiothermic cell lines). Total or partial destruction of monolayers within a 7-d period was considered a positive cytotoxic effect. ECP from *Aeromonas hydrophila* B32 and B51 and *Vibrio anguillarum* RV22 strains were included as positive controls.

Effect of the ECP on the Osmotic Equilibrium of Salmon Erythrocytes

Collection and preparation of blood samples

To avoid possible anaesthetic effects on cellular metabolic activity, fish were stunned by a blow to the head before sam-

TABLE 2. API-ZYM profile of *R. salmoninarum* isolates and ECP. +, positive response; −, negative response.

Strain		Enzymatic activity (numbers indicate API code)								
		1 Control	3 Esterase	4 Esterase lipase	6 Leucine arylamidase	9 Trypsin	10 α-Chymotrypsin	11 Acid phosphatase	12 Phosphomidase	16 α-Glucosidase
ATCC 33209	Live cells	−	+	+	+	+	−	+	+	+
	ECP	−	−	−	−	−	−	−	+	−
416	Live cells	−	+	+	+	+	−	+	+	+
	ECP	−	−	−	−	−	−	−	+	−
417	Live cells	−	+	+	+	+	−	+	+	+
	ECP	−	−	+	−	−	+	−	+	−
420	Live cells	−	+	+	+	+	−	+	+	+
	ECP	−	−	−	−	−	−	−	+	−
421	Live cells	−	+	+	+	+	−	+	+	+
	ECP	−	−	−	−	−	−	−	+	−
422	Live cells	−	+	+	+	−	−	+	+	+
	ECP	−	−	−	−	−	−	−	−	−
426	Live cells	−	+	+	+	−	−	+	+	+
	ECP	−	−	−	−	−	−	−	−	−
444	Live cells	−	+	+	+	+	−	+	+	+
	ECP	−	+	−	−	−	−	−	+	−
466	Live cells	−	+	+	+	+	−	+	+	+
	ECP	−	+	−	−	−	+	−	+	−
R1	Live cells	−	+	+	+	+	−	+	+	+
	ECP	−	−	−	−	−	−	−	+	−

pling. Blood was removed from the caudal vein to heparinized tubes. Plasma osmolarity was measured using a vapour pression osmometer (Wescor 5,000) and the osmolarity of the physiological saline (trout saline) (Bourne and Cossins 1984) was adjusted to that of the plasma by mixing appropriate volumes of salines containing 100 or 200 mM NaCl, pH 7.6 (other constituents were 6 mM KCl, 1 mM $MgSO_4$, 5 mM glucose, and 15 mM imidazole–HCl). Bovine serum albumin (BSA) at 1% (w/v) was included to prevent the slight haemolysis that could occur during long-term storage. Cells were washed at least four times by centrifugation (5 min at 1000 × *g*) followed by resuspension with ice-cold isosmotic saline. The buffy coat and supernatant were discarded. Erythrocytes were finally suspended in isosmotic saline and left for 12–18 h at 4°C.

Measurement of Na^+ and K^+ intracellular levels

Red blood cells were washed four times in trout saline before use and resuspended in saline to give a haematocrit similar to the original blood sample. The assays were carried out by mixing 7.5 mL of suspended erythrocytes with 2.5 mL of each ECP sample. The mixtures were incubated for 8 h. At 2-h intervals an aliquot (250 μL) was taken and the reaction stopped by rapidly washing the cells in ice-cold isotonic $MgCl_2$ solution, pH 7.6 (100–200 mM $MgCl_2$ and 10 mM imidazole), using an Eppendorf centrifuge (5 s at 10 000 × *g*). Cells were washed four times. The final cell pellet was lysed with 0.5 mL of 0.05% (v/v) Triton-X-100 and the protein precipitated with 0.5 mL of 5% (w/v) trichloroacetic acid (2 min at 10 000 × *g*).

The haematocrit (Gelman-Hawksley microhaematocrit centrifuge 8 min at 10 000 × *g*) was routinely monitored during the course of the experiment.

A Perkin-Elmer 603 atomic absorption flame spectrophotometer was used for determination of Na^+ and K^+ intracellular levels (emission mode). Ion concentrations are expressed as millimoles per litre of packed cells. Values were the mean of three separate determinations.

Results

Production of Extracellular Enzymes

The extracellular protein formation and enzymatic activities of our *R. salmoninarum* isolates were evaluated at 15 and 20°C. The enzymatic activity of the ECP using the API-ZYM kit was quite low. Whereas all the bacterial cultures assayed hydrolyzed seven API substrates, the crude extracts displayed only from one to three enzymatic activities (Table 2). Low protein levels were observed in all the ECP samples and the majority of the strains were found to be poor producers of proteolytic enzymes (Table 3). Only the crude extract obtained from two strains, 417 and 466, displayed gelatinase and caseinolytic activities. No haemolytic activity against salmon, trout, or sheep erythrocytes was detected in any of the ECP samples.

No correlation was found between the amount of extracellular protein and protease production, since the highest producers of proteolytic enzymes (417 and 466 isolates) were some of the poorest protein-secreting strains.

Growth temperature did not affect either proteolytic or haemolytic activity.

Toxic Activity of the ECP

The production of cytotoxins was determined in different fish and homoiothermic cell lines. The results showed that, regardless of the origin of the cell line, none of the ECP samples displayed cytotoxic activity after incubation for 1 wk (Table 4).

To evaluate the possible in vivo toxic effects of the extracellular enzymes of *R. salmoninarum*, fingerling rainbow trout were inoculated intraperitoneally with different ECP doses. No mortality was observed after 1 mo when doses ranging from 10 to 20 μg protein/g fish were administered.

Effect of the ECP on the Osmotic Equilibrium of Salmon Erythrocytes

The effect of *R. salmoninarum* exotoxins on the osmotic equilibrium of red blood cells was investigated to establish if

TABLE 3. Toxicity, proteolytic and haemolytic activities of ECP from *R. salmoninarum* isolates.

Strain	Total protein (μg/mL)	Proteolytic activity (units/mL) on:			Haemolytic activity (HU/mg protein) in:[c]			Fish mortality (no. dead/ no. tested)[d]	Inoculated dose (μg protein/g fish)
		Azocoll[a]	Casein[b]	Gelatin[b]	Salmon	Trout	Sheep		
ATCC 33209	400	0.2	0	0	<2	<2	<2	0/5	20
416	200	1.3	0	0	<2	<2	<2	0/5	10
417	250	6	84	68.5	<2	<2	<2	0/6	12.5
420	250	0.1	0	0	<2	<2	<2	0/4	12.5
421	275	0.3	0	0	<2	<2	<2	0/5	13.75
422	250	0	0	0	<2	<2	<2	0/4	12.5
426	300	0	0	0	<2	<2	<2	0/6	15
444	250	0.6	0	0	<2	<2	<2	0/5	12.5
466	250	1.2	75	57.5	<2	<2	<2	0/5	12.5
R1	275	0.4	0	0	<2	<2	<2	0/6	13.75

[a]One proteolytic unit was defined as a change in the A_{520} of 1.0 after a 30-min assay at 37°C.
[b]One unit of caseinase or gelatinase activity was defined as that which produced a zone of clearing equal in area to that produced by 1 μg of trypsin.
[c]One haemolytic unit (HU) was expressed as the reciprocal of the highest dilution of ECP producing complete haemolysis.
[d]Rainbow trout were inoculated intraperitoneally with 0.15 mL of each ECP.

TABLE 4. Absence of cytotoxic activity of *R. salmoninarum* ECP on tissue cultures. +, positive response; −, negative response.

Strain	Fish cell line						Homoiothermic cell line	
	CHSE-214	RTG-2	FHM	ECP	BF-2	BB	L-929	Hela
R. salmoninarum								
ATCC 33209	−	−	−	−	−	−	−	−
416	−	−	−	−	−	−	−	−
417	−	−	−	−	−	−	−	−
420	−	−	−	−	−	−	−	−
421	−	−	−	−	−	−	−	−
422	−	−	−	−	−	−	−	−
426	−	−	−	−	−	−	−	−
444	−	−	−	−	−	−	−	−
466	−	−	−	−	−	−	−	−
R1	−	−	−	−	−	−	−	−
Controls								
A. hydrophila								
B32	+	+	+	+	+	+	+	+
B51	+	+	+	+	+	+	+	+
V. anguillarum								
RV22	+	+	+	+	+	+	+	+

this contributed to the reported decline in the number of circulating erythrocytes. No significant changes were observed in the intracellular concentration of Na^+ and K^+ between the cells incubated with *R. salmoninarum* ECP and the control erythrocytes in saline.

Discussion

It is known that extracellular enzymes produced by some fish pathogenic bacteria such as motile *Aeromonas* (Thune et al. 1986; Santos et al. 1988), *Aeromonas salmonicida* (Hastings and Ellis 1985; Sakai 1985), and *Vibrio anguillarum* (Inamura et al. 1984; Kodama et al. 1985) are implicated in the pathogenesis of the diseases caused by these microorganisms. Until now, however, few investigations of this nature have been carried out with *R. salmoninarum*.

In this study, little production of extracellular proteins was observed for all the strains. In addition, most of the isolates were poor producers of proteolytic enzymes and only two strains displayed caseinolytic and gelatinase activities.

Erythrocytes from rainbow trout, salmon, and sheep were employed in the haemolytic assays. Interestingly, although it has been reported that some *R. salmoninarum* strains are β-haemolytic in MHA-C with rabbit blood added (Bruno and Munro 1986b), none of the poikilothermic and homoiothermic erythrocytes tested were sensitive to the extracellular enzymes of our isolates. Possible explanations for this difference could be (1) the distinct kind of red blood cells employed, (2) loss of haemolytic activity during the storage process or (3) the lack of secretion of haemolysins by the bacteria. The results of the API-ZYM seem to support the last theory, since the live cells possess higher enzymatic activity than the ECP. This suggests that many of these activities could be associated with the cell envelope rather than being released into the medium.

Recent reports have demonstrated the presence of *R. salmoninarum* soluble antigens in fish experimentally infected with live cells (Turaga et al. 1987) and that the ECP produced in vitro by the *R. salmoninarum* type strain ATCC 33209 is lethal to fingerling Atlantic salmon (Shieh 1988). However, we have inoculated our crude extracts into fingerling rainbow trout without inducing mortality, although the doses were similar to those reported to be lethal in other fish pathogens (Kanai and Wakabayashi 1984; Kodama et al. 1985; Santos et al. 1988). Moreover, the large amount of protein reported by Shieh (1988) needed to kill fish (320 μg protein/g fish) indicates that *R. salmoninarum* ECP is not a determinative factor in fish mortalities from BKD.

The production of cytotoxins was evaluated in six fish cell lines and two homoiothermic cell cultures. Interestingly, regardless of the origin of the cell lines used, no cytotoxic effect was detected after incubation for 1 wk. As reported in other bacteria pathogenic for fish (Toranzo et al. 1987; Santos et al. 1988), cytotoxins are not essential in the pathogenesis of BKD.

Previous studies have suggested that bacterial toxins could be responsible for the significant decline in red cell count, haematocrit, and haemoglobin with an associated splenomegaly observed in fish infected with BKD (Suzumoto et al. 1977; Aldrin et al. 1978; Kimura 1978; Bruno and Munro 1986c). However, in the present study we have demonstrated that the ECP failed to show either haemolytic activity or capacity to alter the osmotic equilibrium of fish erythrocytes. Therefore, further studies are necessary to determine the mechanisms involved in the observed changes in blood parameters.

Although we failed to demonstrate toxicity in vivo of ECP samples, we cannot rule out that production of some enzymes may contribute to both the weakening of fish during the course of disease and to bacterial survival.

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Large-Scale Escapes of Farmed Atlantic Salmon (*Salmo salar*) into Norwegian Rivers Threaten Natural Populations

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Gausen, D., and V. Moen. 1991. Large-scale escapes of farmed Atlantic salmon (*Salmo salar*) into Norwegian rivers threaten natural populations. Can. J. Fish. Aquat. Sci. 48: 426–428.

We present data on Atlantic salmon (*Salmo salar*) which show that escaped farmed salmon enter Norwegian rivers in great numbers. Escaped farmed salmon comprised a substantial proportion of the mature salmon present on the spawning grounds in autumn. A potential for large-scale genetic introgression thus exists. The sex ratios, length distributions, and times of ascent of wild and farmed salmon differed significantly, reflecting major variations in biology and behavior. High proportions of farmed salmon (>20%) were found only in rivers having fish farms situated closer than 20 km from the outlet.

Les auteurs présentent des données sur le saumon de l'Atlantique (*Salmo salar*) qui révèlent que les saumons d'élevage en liberté remontent les rivières norvégiennes en grand nombre. Les saumons d'élevage en liberté représentent un important pourcentage des saumons matures présents dans les frayères à l'automne. Il existe donc un potentiel élevé d'introgression génétique à grande échelle. La nette différence de la proportion des sexes, de la distribution des longueurs et du moment de la remonte des saumons sauvages et des saumons d'élevage exprime d'importantes variations de la biologie et du comportement. Des pourcentages élevés de saumons d'élevage (> 20%) ont été notés que dans les rivières abritant des pisciculture à moins de 20 km de l'embouchure.

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The rapid advance of the aquaculture industry had led to an increased risk of spreading genetically modified organisms among natural populations. Norway is a leading country in salmon farming. In 1988 the number of Atlantic salmon smolts (*Salmo salar*) stocked into sea cages was about 100 times the average number of wild salmon migrating into coastal waters each year (estimated to be 700 000 individuals). The number that escape from sea cages is thought to be considerably greater than the total number of wild fish in that approximately 700 000 farmed fish escaped from one area alone (in the middle coastal region of Norway) during a single episode in the winter of 1988–89. Escapes result from various kinds of accidents, including cage damage caused by seals, mink, and otters, general wear and tear, and stranding of cages in bad weather (often due to loss of moorings). The latter is thought to account for the majority of large-scale escapes.

In Norway, farmed salmon have been intensively bred for 15 yr. The breeding programme is aimed at developing domesticated strains with favorable production traits, such as rapid growth, high age at maturity, docility, and resistance to disease.

Many biological traits in wild salmon are population specific (Saunders 1981). Crossbreeding between farmed and wild salmon in nature may lead to the loss of genetically determined characteristics of local adaptive value to wild populations. Several authors have documented with other species of salmonids the negative effects of natural crossbreeding between introduced and indigenous strains (Taggart and Ferguson 1986; Nickelson et al. 1986; Altukhov and Salmenkova 1987; Guyomard 1989). Some work has been done on reproductive success (Chilcote et al. 1986), survival (Reisenbirchler and McIntyre 1977), and "homing" (Bams 1976) of introduced salmonids, all of which showed a poorer performance than the indigenous fish.

Most salmon populations in Norway are small and therefore highly vulnerable to genetic deterioration. More than half the rivers have fewer than 100 spawning fish annually (Ståhl and Hindar 1988). The total number of salmon spawning in autumn is estimated to be about 100 000 (due to heavy exploitation), distributed among 400 rivers (Ståhl and Hindar 1988).

The aim of this investigation was to obtain basic information about the occurrence of escaped farmed salmon in a large number of salmon rivers throughout Norway.

Materials and Methods

A total of 3700 salmon were examined. We sampled information on the fishes' length, weight, sex, and time and place for capture in 54 rivers (Fig. 1). Wild and farmed specimens were identified according to their external characteristics and from scale analysis (Lund et al. 1989). Samples were taken during the period May–November 1988 and from entire rod and net catches.

Results and Discussion

Farmed salmon were found in 38 (70%) of the investigated rivers. Farmed salmon accounted for 15% of the total sample

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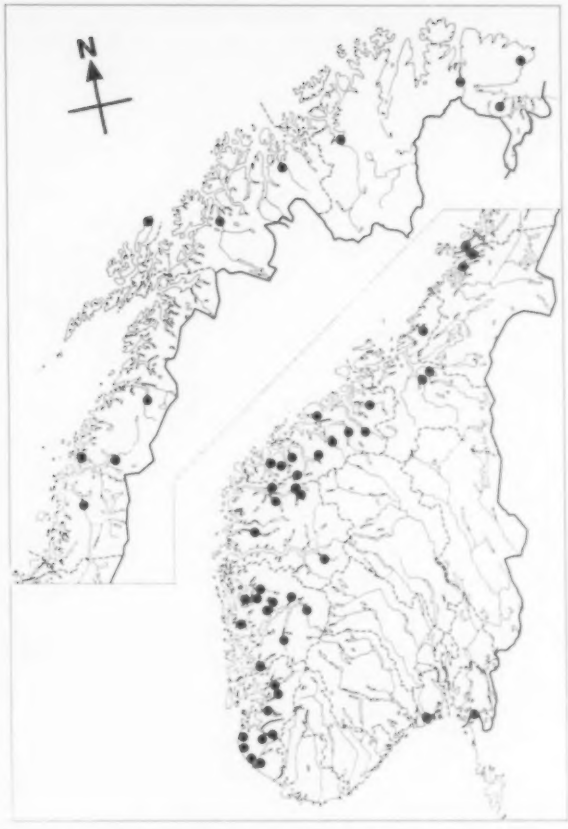

FIG. 1. Locations of the 54 watercourses investigated in northern (upper part) and southern (lower part) Norway.

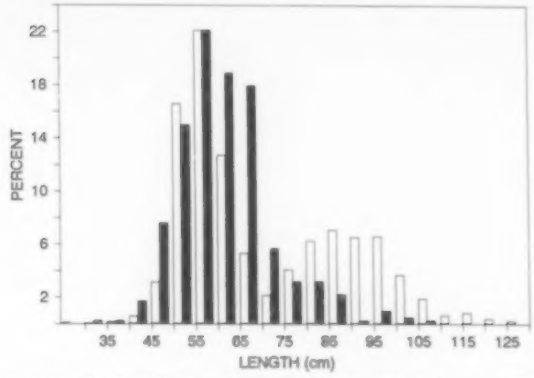

FIG. 2. Total length distribution of wild salmon (white bars, $n = 2649$) and farmed salmon (black bars, $n = 407$) from all rivers.

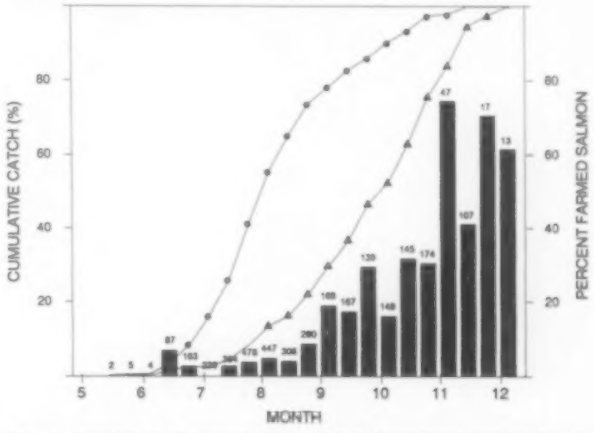

FIG. 3. Proportion of farmed salmon (black bars) (total number in the sample is indicated on top of each bar) and cumulative catch of wild salmon (●) and farmed salmon (▲) throughout the sampling period.

in southern Norway (2741 individuals sampled), but only 1% in northern Norway (959 individuals sampled). This reflects the considerably greater volume of production of farmed salmon in southern Norway compared with northern Norway. The respective values for the two regions in 1988 were 60 000 and 15 000 tonnes. The natural production of wild salmon in the two regions is about equal and the coastlines are of approximately equal length.

The length distribution of the wild salmon was bimodal, corresponding to fish that have spent one or several winters at sea (Fig. 2). The length distribution of the escaped farmed salmon was unimodal, and most were fish that had spent one winter in salt water. This may be attributed to the facts that the farming industry produces predominantly one-sea-winter fish and that few salmon will become mature and enter the rivers at an earlier sea-age. Multi-sea-winter farmed fish have spent at least one winter at sea since their escape and subsequently must have experienced a higher total mortality. The scarcity of large escaped farmed salmon indicates a high mortality at sea. The length distribution of the wild salmon varied between rivers, reflecting local variations in growth conditions and life history. No such differences existed in farmed salmon, which may be either larger or smaller than local wild populations. This may influence the competitive relationship between the escaped farmed salmon and the wild salmon in individual rivers.

The sex distribution of wild salmon (52% males) did not differ significantly from a 50/50 distribution ($p > 0.05$). Males predominated (68%) ($p < 0.001$) among the farmed salmon. This may be attributed to the fact that farmed males have a tendency to become sexually mature, and therefore ascend the rivers, at an earlier age than the females. Nearly all the salmon examined had either partially or fully developed gonads. In Norway, most salmon spawn between the middle of October and the end of November (Heggberget 1988). During this period a major proportion of all the fish sampled, both farmed and wild, had ovulated eggs and running milt, a state which occurs shortly before spawning. Physiologically, farmed salmon therefore seem fully capable of spawning, either among themselves or with the wild salmon.

Farmed salmon ascent rivers about 2 mo later than the wild salmon as indicated by the cumulative catch curves (Fig. 3). Therefore an exponential increase in the proportion of farmed salmon occurred between June and November ($p < 0.01$; $r^2 = 0.884$, $n = 18$) (Fig. 3), i.e. the migratory behavior of the farmed salmon differs from that of the wild salmon. One hypothesis is that a further advanced maturation is necessary before the farmed salmon will enter the rivers. The underlying causes are most probably to be sought in the fact that the farmed salmon originate from an artificial environment and selection regime.

While contained in the sea cages, the fins of farmed salmon often become eroded and the fish may also have reduced muscle

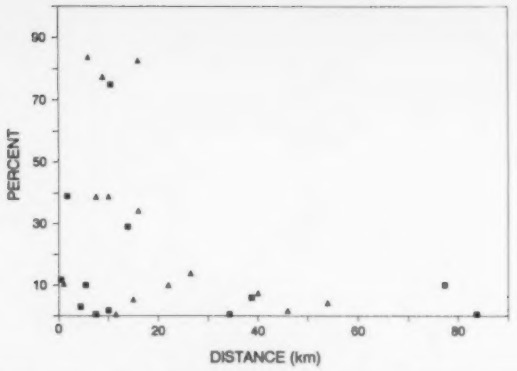

FIG. 4. Proportion of farmed salmon in individual rivers plotted against distance to the nearest cage culture (▲) and smolt farm (■) in autumn (18 August to 30 November). Only rivers with more than 20 fish sampled are included.

power and stamina because of reduced exercise compared with the wild salmon. This could reduce its capability to overcome major obstacles such as waterfalls. The proportion of farmed salmon downstream from a waterfall in the River Suldalslågen was 26% (46 fish examined) compared with only 1% (151 fish examined) upstream from the waterfall. A waterfall in the River Oselva apparently posed less of an obstacle because the respective proportions here were 83% below (122 fish sampled) and 62% above (53 fish sampled) the waterfall.

Rivers lying within a short distance of a fish farm (<20 km) may or may not have a large proportion of farmed salmon (Fig. 4). However, high proportions of farmed salmon (>20%) were never found in rivers at a distance greater than 20 km from the nearest cage culture or smolt farm. This may indicate that escaped farmed salmon either have a limited ability to spread or that mature fish return to the areas from which they escaped. Analysis of recapture statistics for tagged, farmed salmon released in the sea indicates that the latter may sometimes be the case (Hansen et al. 1989).

Escaped farmed salmon comprised a substantial proportion of the mature salmon present on the spawning grounds in October and November (Fig. 3). A potential for large-scale introgression of exogenous genetic material into native populations thus exist. If this situation should persist for some years, it could mean a total breakdown of the stock structure of the Atlantic salmon in Norway. This would obliterate the genetic differences between salmon in different rivers, leading to loss of traits of local adaptive value to wild populations. There is also a great danger that some escaped farm salmon may carry diseases which will spread to many rivers. In recent years there has been an increased occurrence of furunculosis in salmon rivers in the middle region of Norway. This disease was not recorded previously in this region.

At present we have no data on the reproductive success of ecaped farmed salmon, and little knowledge exists about their general behavior and their competitive relationship with wild salmon. Further research into these topics will yield clues as to the future impacts of the escaped farmed salmon upon the wild salmon populations. However, our investigation indicates that the problem is already far advanced and that it is necessary to take preventive measured now to safeguard the local salmon populations. We feel that the problem lies in the actual escape of the farmed salmon and that the only solution is to ensure that escapes do not occur.

Acknowledgements

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A Method of Separating Offspring from Farmed and Wild Atlantic Salmon (*Salmo salar*) based on Different Ratios of Optical Isomers of Astaxanthin

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Lura, H., and H. Sægrov. 1991. A method of separating offspring from farmed and wild Atlantic salmon (*Salmo salar*) based on different ratios of optical isomers of astaxanthin. Can. J. Fish. Aquat. Sci. 48: 429–433.

A method of separating eggs and alevins from wild and farmed Atlantic salmon (*Salmo salar*) is described, using high-performance liquid chromatography (HPLC) to trace optical isomers of astaxanthin commonly occurring in the diet of both wild and farmed salmon. The proportions of isomers in eggs and alevins of farmed salmon, fed synthetic astaxanthin, differ highly from those of wild fish. The method can be used as a tool to determine the spawning success of escaped farmed female Atlantic salmon.

On décrit une méthode d'identification des oeufs et des alevins vésiculés du saumon de l'Atlantique (*Salmo salar*) sauvage et d'élevage. La chromatographie en phase liquide moderne permet de dépister les isomères optiques de l'astaxanthine communément présente dans la régime alimentaire des saumons sauvages et des saumons d'élevage. Le pourcentage d'isomères chez les oeufs et les alevins vésiculés de saumons d'élevage nourris d'aliments contenant de l'astaxanthine synthétique varie grandement par rapport au pourcentage noté chez les saumons sauvages. La méthode décrite peut être utilisée pour déterminer le succes de la reproduction des saumons femelles d'élevage en liberté.

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In recent years, farming of Atlantic salmon (*Salmo salar*) has become a large industry in Norwegian coastal waters. The production in 1989 was reported to be 115 000 metric tons. Given an average individual weight of 4 kg, 29 million salmon were produced. In contrast, the total spawning population of wild Atlantic salmon in Norway 1986 was estimated at 100 000 individuals (Ståhl and Hindar 1988) distributed among 400–500 stocks. Due to the method of production (i.e. net pens), some salmon escape from farms, later maturing and migrating into rivers, presumably to spawn. An extensive registration of farmed fish in catches was reported for several rivers in 1988 from August to November by Moen and Gausen (1989). They showed that escaped salmon contributed 26% (average in 30 rivers) of the mature salmon in Norwegian rivers, the highest value being in the River Os where 77% (N = 178) of the running salmon were of farmed origin. Since the 1970s there has been a Norwegian breeding program, providing more than 90% of the smolts to Norwegian fish farms. Within this program, fish have been heavily selected for economically important traits, e.g. growth rate, delayed maturation, disease resistance, and flesh quality. Due to the high number of escaped farmed salmon, there is a potential risk of genetic contamination of the wild salmon by means of gene flow, possibly reducing the fitness of locally adapted populations (Ståhl 1987; Ståhl and Hindar 1988). For the conservation of wild salmon, knowledge of the extent of the gene flow from farmed salmon to wild stocks is required. Ideally this should incorporate reproductive success for both populations, but one obstacle has been the difficulty of assessing spawning success for the two groups.

Here we introduce a new method of identifying eggs and alevins from farmed and wild salmon based on the proportions of the three optical isomers of astaxanthin. The method is based on the fact that farmed salmon are fed carotenoids throughout the sea period to attain the attractive pink colour of salmon flesh. Earlier, synthetic canthaxanthin was used as the source of pigmentation in the farming industry, and detection of this carotenoid has been proposed as a method of separating farmed and wild individuals (Craik and Harvey 1987). However, during the last 3 yr, astaxanthin (3,3′-dihydroxy-β,β-caroten-4,4′-dione), which is also the main natural pigment of wild salmon (Goodwin 1984), has been the main carotenoid in use in the Norwegian fish farming industry. Approximately 90–95% of the carotenoid is of synthetic origin, the rest coming from crustacean waste products (Torrisen 1989), mainly *Pandalus borealis*. Astaxanthin exists in three optical isomers (Fig. 1), and synthetic astaxanthin is a mixture of them in the following ratio: (3R, 3′R):(3R, 3′S):(3S, 3′S) = 25:50:25 (Vecchi and Müller 1979; Storebakken et al. 1985). This was confirmed by a high-performance liquid chromatography (HPLC) analysis of the astaxanthin (carophyll pink) produced by F. Hoffmann-La Roche & Co. Ltd., currently the source of all the synthetic astaxanthin used in the Norwegian farming industry.

Salmon cannot synthesize astaxanthin (Goodwin 1984). Studies on Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) show that there is no selective absorption of the different isomers and no epimerization at the chiral centers C-3 and C-3′ (Storebakken et al. 1985; Foss et al. 1984). Therefore, flesh contents of optical isomers reflect the diet of the animal. Further, flesh of wild Atlantic salmon is reported to have a different isomeric distribution (Shiedt et al. 1981) from that found in salmon fed synthetic astaxanthin (Storebakken et al. 1985) (Table 1). Therefore, we expected to find differ-

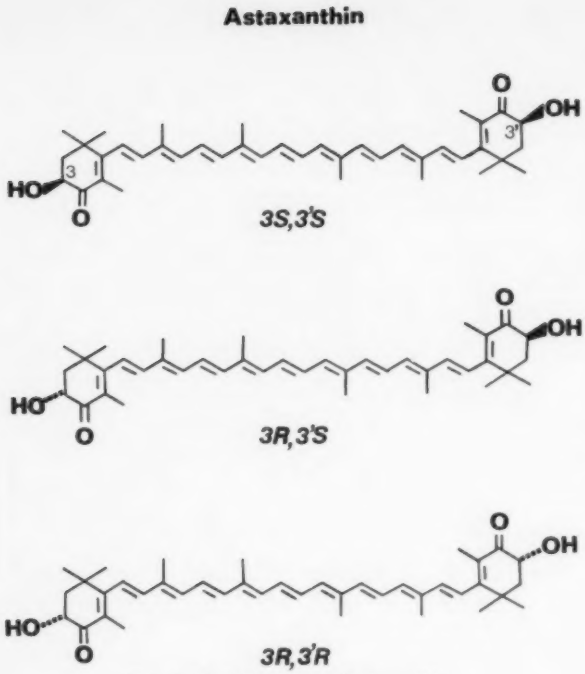

FIG. 1. Optical isomers of astaxanthin.

TABLE 1. Reported isomeric ratios in flesh of wild and farmed Atlantic salmon. The farmed salmon were fed an experimental diet containing synthetic astaxanthin. Note that the wild fish are two from Scotland and one each from Ireland and Norway.

	Optical isomer			
	(3R, 3'R)	(3R, 3'S)	(3S, 3'S)	*N*
Farmed[a]	25.8	42.7	31.9	4
Wild[b]	14.6 ± 2.36	4.8 ± 0.68	80.6 ± 2.34	4

[a]Storebakken et al. (1985).
[b]Schiedt et al. (1981).

ences in the isomeric ratios in farmed escaped Atlantic salmon and feral fish. We also expected that this difference would be found in ova and alevins of the two types of salmon, since astaxanthin is transferred from flesh to gonadal tissues. In analyzing ova and alevins excavated from spawning redds, isomeric ratios can be used to distinguish between the offspring of farmed and wild fish, thereby making it possible to assess spawning rate and success of escaped salmon.

Materials and Methods

During autumn 1988 and spring 1989, eggs and alevins from the following three Norwegian stocks of farmed salmon were sampled at random: (1) newly fertilized ova from Blom Fishfarm (<100 degree days), (2) eyed ova from Vanylven parent fish station (300 degree-days), and (3) newly hatched alevins from Erfjord parent fish station (470 degree-days). These three stocks will be referred to as farmed standards. At Blom Fishfarm they rear their own parent fish and feed the fish moist pellets containing a mixture of synthetic astaxanthin and astaxanthin from shrimp waste (*P. borealis*). The other two stocks are both descendants from the Norwegian breeding program, but are reared in different parts of Norway and fed on a dry pellet diet containing synthetic astaxanthin.

Eggs and alevins from wild salmon were sampled at random from three different hatcheries which provide alevins for enhancement of the local salmon populations in the Rivers Hå, Ogna, Etne, and Arna. All parent fish used in these hatcheries were caught in the local rivers when returning from their natural feeding areas in the North Atlantic Ocean. New wild parent fish are caught every year. The wild fish were separated from farmed escapees by means of external morphology (Hansen et al. 1987; Lund et al. 1989). Erroneous classification of wild salmon by this method is negligible. These samples will be referred to as wild standards.

Further, eggs and alevins from natural spawning redds in the Rivers Arna, Os, and Etne were sampled randomly during spring 1989. Redds were excavated and eggs and alevins collected downstream in a net (Briggs 1953). All eggs and alevins were kept deep-frozen (−20°C) until analysis.

The optical isomers of astaxanthin in single eggs and alevins were separated via (−)-camphanic acid diesters by means of HPLC (Vecchi and Müller 1979) using Spherisorb CN (5 μm) as the stationary phase. Column length was 100 mm and internal diameter was 3 mm. In addition, a guard column containing Spherisorb CN (30–40 μm), length 10 mm and diameter 2.1 mm, was used. Hexane – isopropyl acetate – acetone (76:17:7) was used as the mobile phase (flow 0.4 mL/min). Peaks were detected at 478 nm.

Extraction of carotenoids from eggs or alevins and sample preparation were carried out as follows. One egg or alevin was homogenized in 1.5 mL of water and extracted twice with 2 mL of ether – methanol (2:1). The combined ether phases were washed with water (0.5 mL) and dried under a N_2 atmosphere. Twenty milligrams of camphanic acid chloride was added together with 200 μL of dry pyridine and left for 1 h at 0°C. Half a millilitre of water was added and the mixture extracted twice with 2 and 1 mL of ether, respectively. The combined ether phases were again washed with water (0.5 mL) and dried under a N_2 atmosphere. Finally the sample was dissolved in 500 μL of HPLC solvent (mobile phase). The presence of pyridine in the reaction mixture favours esterification by the nucliophilic route (March 1985, p. 347) and thus avoids uncertainties with regard to the stereochemical integrity of the results. One trained person can do 30–40 samples a day.

During development from egg to fry, much of the free astaxanthin in a specimen is esterified to mono- and diesters (Glover et al. 1952; Craik and Harvey 1986) and transferred to the integument. However, assuming that the natural esterification in alevins does not discriminate with respect to the stereochemistry of astaxanthin, the analysis of free astaxanthin will give a true picture of the isomeric distribution.

Atlantic salmon occur in sympatry with brown trout (*Salmo trutta*), Arctic char (*Salvelinus alpinus*), and probably rainbow trout, the latter escaping from farms (Moen and Gausen 1989). Eggs and alevins sampled from redds were identified as to species by means of phosphoglucose isomerase (PGI) zymograms from isoelectric focusing. PGI zymograms are considered species diagnostic keys in identifying Norwegian stream-dwelling salmonids (Mork and Heggberget 1984). Hybridization between species is negligible in Norwegian rivers (Ståhl and Hindar 1988). Isoelectric focusing was performed in a LKB Ampholine PAGplate ready-to-use polyacrylamide gel (pH 3.5–9.5,

TABLE 2. Proportions of optical isomers of astaxanthin in eggs or alevins of farmed and wild Atlantic salmon. One egg or alevin per female was analyzed. *N* = number of females. Standards refer to the two groups sampled from hatcheries. Values are given as mean ± SD.

	Optical isomer			
	(3R, 3′R)	(3R, 3′S)	(3S, 3′S)	*N*
Farmed standards				
Blom	20.55 ± 0.82	47.00 ± 1.94	32.45 ± 1.29	6
Vanylven	20.45 ± 0.77	46.73 ± 1.20	32.85 ± 1.70	4
Erfjord	20.40 ± 0.61	46.83 ± 1.16	32.78 ± 1.60	4
Total	20.5 ± 0.69	46.9 ± 1.45	32.7 ± 1.39	14
Range	19.2 – 21.3	45.3 – 50.5	30.3 – 34.3	
Wild standards				
Etne	13.3	3.8	82.9	1
Arna	10.51 ± 0.57	3.54 ± 0.50	86.05 ± 1.06	2
Ogna	14.10 ± 3.54	4.20 ± 0.57	81.70 ± 1.55	2
Hå	11.07 ± 1.55	3.51 ± 0.90	85.44 ± 1.55	7
Total	11.7 ± 2.07	3.6 ± 0.75	84.7 ± 2.36	12
Range	9.3 – 16.6	2.3 – 4.9	78.8 – 87.2	

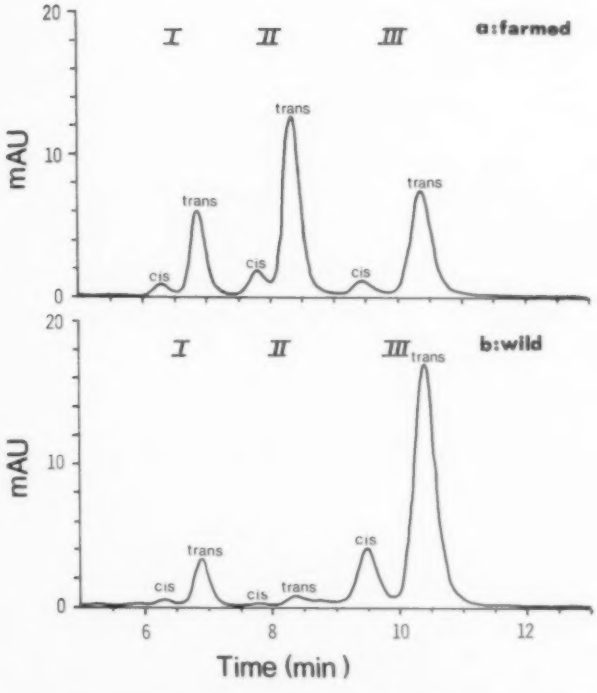

FIG. 2. HPLC separation of diastereomeric esters of (−)-campanic acid with astaxanthin from an egg of (a) farmed and (b) wild Atlantic salmon, respectively. The peaks of the optical isomers (*cis* and *trans*) I: (3R, 3′R), II: (3R, 3′S), and III: (3S, 3′S) are easily distinguishable. It is of no interest in our study to distinguish between the *cis* and *trans* forms of the single isomers. Thus, the isomeric ratios are calculated as the proportion of the total area of astaxanthin (−)-campanic diesters under each isomer's *cis* and *trans* peak.

TABLE 3. Optical isomer proportions of astaxanthin in eggs or alevins from spawning redds and classification of the spawning female according to standards. E = eggs; A = alevins.

Female No.	Optical isomer (3R, 3′R)	(3R, 3′S)	(3S, 3′S)	Eggs and alevins	Classification
Arna					
1	20.7	43.5	35.8	E	Farmed
2	19.3	39.3	41.5	E	Farmed
3	20.5	47.6	31.9	E	Farmed
Etne					
1	21.5	43.3	35.2	A	Farmed
2	16.8	2.6	80.6	A	Wild
3	14.7	5.4	79.9	A	Wild
4	16.3	5.9	77.9	A	Wild
5	12.2	4.4	83.4	A	Wild
6	17.3	4.3	78.3	A	Wild
7	21.7	46.0	32.3	A	Farmed
8	16.0	5.6	78.4	E	Wild
9	17.6	4.6	77.9	A	Wild
10	13.5	4.2	82.4	A	Wild
11	16.0	6.3	77.7	A	Wild
12	16.9	6.0	77.1	A	Wild
13	15.6	5.2	79.3	A	Wild
Os					
1	14.0	3.9	82.1	E	Wild
2	17.8	33.0	49.2	A	Farmed
3	10.1	4.0	85.9	A	Wild
4	11.8	4.7	83.6	E	Wild
5	12.0	4.7	83.3	A	Wild
6	21.8	5.2	73.0	A	Wild
7	19.7	44.7	35.5	E	Farmed
8	20.1	46.5	33.5	E	Farmed
9	21.1	45.9	33.0	E	Farmed

TABLE 4. Optical isomer proportions of astaxanthin in eggs or alevins from spawning redds. Pooled data from Table 3. Salmon classified as wild are from the Rivers Etne and Os; all farmed salmon are combined. Values are given as mean ± SD, with ranges below.

	Optical isomer			
	(3R, 3′R)	(3R, 3′S)	(3S, 3′S)	*N*
Wild Etne	15.72 ± 1.66	4.95 ± 1.07	79.35 ± 2.04	11
Range	12.2 – 17.6	2.6 – 6.3	77.1 – 83.4	
Wild Os	13.94 ± 4.61	4.50 ± 0.54	81.58 ± 4.99	5
Range	10.1 – 21.8	3.9 – 5.2	73.0 – 85.9	
Farmed total	20.27 ± 1.22	43.31 ± 4.57	36.43 ± 5.58	9
Range	17.8 – 21.7	33.0 – 47.6	31.9 – 49.2	

V_{max} 1600 V, 150 mA, 19 W at 4°C). Total focusing time was 120 min. Only salmon redds are considered in this study.

Results and Discussion

Eggs and alevins of farmed and wild standards fall into two distinct groups with respect to proportions of optical isomers (Table 2). The two groups can be easily separated (Fig. 2). There is a striking similarity in isomeric ratios for the three farmed stocks used as standards (Table 2), with virtually no variation.

Proportions of isomers in eggs and alevins from different spawning redds in the Rivers Arna, Os, and Etne clearly resemble those in one or the other of the standard groups. The classification of fish belonging to either the farmed or the wild group is based on the fact that there is no overlap in ranges between the two standard groups (Tables 3 and 4).

The reported values from fish flesh (Table 1) correspond well with our data from eggs and alevins, suggesting a proportional transfer of the isomers from flesh to female gonadal tissues (see Ando 1986).

The high proportion of the (3R, 3′S) isomer in synthetic astaxanthin of commercial salmon food is reflected in farmed fish whereas in wild salmon this component is 10 times lower. Food intake of wild fish is much reduced as they reach the coast (L. P. Hansen, NINA, Tungasletta 2, N-7004 Trondheim, Norway, pers. comm.) so it is unlikely that wild fish eat pellets containing synthetic astaxanthin under farm pens. In contrast, escaped salmon may switch to natural food before entering the rivers, and it is expected that this affects the mixture of isomers in such individuals. Mean values of the isomers (3R, 3′S) and (3S, 3′S) found in spawned eggs and alevins classified as offspring from escapees differ significantly from the farmed standards (isomer (3R, 3′S): $t = 2.762$, df = 21, $p < 0.01$, one-tailed; isomer (3S, 3′S): $t = 2.416$, df = 21, $p < 0.025$, one-tailed) and are skewed towards the wild standards (Tables 2 and 4). This indicates intake of astaxanthin from natural food before entering the rivers. We suggest that the change in isomeric ratios towards the wild fish is related to the time since escape. Intake of natural food will cause an increased variation in isomer proportions of the escaped fish compared with the farmed standards, as is shown in the increased ranges for these two groups (Tables 2 and 4). Note that the difference in isomeric ratios between escaped and wild fish is still striking.

Further research will show for how long escaped fish will contain traceable synthetic components. Also, it is important to know if fish escaping at an early age will be detectable later as escapees. It is uncertain for how long the carotenoid can be traced in salmon offspring. By way of example, we found detectable amounts of astaxanthin in alevins which had almost completely absorbed the yolk sac. However, since fry metabolize and esterify most of the astaxanthin (Craik and Harvey 1986), there might be problems in detecting unesterified carotenoid in fry.

The fact that shrimp wastes are sometimes added to commercial food does not affect the isomer mixture in farmed salmon, since shrimps (*P. borealis*) show similar proportions of isomers to synthetic astaxanthin (Renstrøm et al. 1981). However, *P. borealis* does not occur in the diet of wild salmon (Hansen and Pethon 1985). Six of the farmed females used as standards had been fed some shrimp wastes (Blom Fish-farm), but they did not differ in isomeric proportions compared with the eight other farmed standards (Table 2).

Our results from wild fish (Tables 2 and 4) correspond well with isomeric ratios reported by Schiedt et al. (1981) (Table 1). Their values for Atlantic salmon are from four individual fish, two from Scotland and one each from Ireland and Norway. They also reported isomeric ratios in three species of *Oncorhynchus* which fall within the range for wild salmon in our study. This illustrates the accuracy of our method and shows that it can be applied to other salmon species, and probably other salmonids as well, given that farmed fish are fed synthetic astaxanthin. Feeding salmonids synthetic astaxanthin can thus be used as a marking method, making it possible to assess spawning success, since the mark is transferred from mother to offspring.

Our study further shows that there are significant differences between alevins of wild fish in the River Etne and the wild standard fish from the River Hå in isomer (3R, 3′R) ($t = 5.938$, df = 16, $p < 0.001$) and (3S, 3′S) ($t = 6.731$, df = 16, $p < 0.001$), respectively (Tables 2 and 4). As discussed above, this is due to differences in preferred prey or regional differences in production of isomers eaten by the salmon during their migration. It is reported by Foss et al. (1987) that the copepod *Calanus finmarchicus* had isomeric ratios similar to wild salmon ((3R, 3′R):(3R, 3′S):(3S, 3′S) = 14:3:83). Calanoid copepods are the dominating crustacean zooplankton in the main feeding area of Atlantic salmon and are likely to be the source of astaxanthin. However, details in differences between populations have to be further investigated.

In conclusion, our method can be used to assess spawning rate and success of farmed salmon. All salmon falling outside the range of wild fish should be classified as escapees. Our method will classify most salmon as farmed or wild where synthetic astaxanthin is fed throughout pen rearing, as in Norway. Even then, the method will give conservative estimates because salmon that escape as smolts or presmolts cannot be traced, since no astaxanthin is added to the food during the freshwater phase. The method is a potentially important tool for salmon management.

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Food Consumption and Bioenergetics of Young-of-the-Year Walleye (*Stizostedion vitreum vitreum*): Model Predictions and Population Density Effects

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Fox, M. G. 1991. Food consumption and bioenergetics of young-of-the-year walleye (*Stizostedion vitreum vitreum*): model predictions and population density effects. Can. J. Fish. Aquat. Sci. 48: 434–441.

I compared in situ growth and food consumption estimates of young-of-the-year walleye (*Stizostedion vitreum vitreum*) reared at three different densities in outdoor ponds with predictions of a bioenergetic model for walleye (Kitchell et al. 1977. Res. Board Can. J. Fish. 34: 1922–1935), and used the model to determine whether fish population density affects growth efficiency or metabolism. Maximal walleye growth rate was adequately predicted by model simulations. Consumption estimates were 40% lower than predicted, but predictions were improved when the respiration equation of Minton and McLean (1982. Can. J. Fish. Aquat. Sci. 39: 1396–1403) was substituted in the model. There was no evidence that juvenile walleye have higher consumption or respiration rates than those predicted by allometric models based on larger fish. Walleye in low-density ponds had a higher growth efficiency and active metabolic rate than those reared at lower densities, but the difference was not statistically significant.

J'ai comparé des estimations de la croissance *in situ* et de la consommation d'aliments chez les dorés (*Stizostedion vitreum vitreum*) de la classe de l'année, élevés en trois densités différentes dans des étangs extérieurs, avec les prévisions d'un modèle bioénergétique pour le doré (Kitchell *et al.* 1977. J. Fish. Res. Board Can. 34: 1922–1935). J'ai employé le modèle pour déterminer si la densité des populations de poissons influait sur l'efficacité de la croissance ou le métabolisme. Des prévisions pertinentes du taux de croissance maximal des dorés ont été établies par des simulations du modèle. Les évaluations de la consommation étaient environ 40 % inférieures aux prévisions, mais celles-ci ont été améliorées lorsque l'équation de Minton et McLean (1982. Can. J. Fish. Aquat. Sci. 39 : 1396–1403) pour mesurer la respiration a été remplacée dans le modèle. Rien n'indiquait que les dorés juvéniles aient eu des taux de consommation ou de respiration plus élevés que ceux prévus par des modèles allométriques à partir de plus gros poissons. Les dorés des étangs à faible densité avaient une efficacité de croissance et un taux d'activité métabolique supérieurs à ceux des dorés élevés en faibles densités, mais la différence n'était pas significative statistiquement.

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Bioenergetic models have been successfully used to account for seasonal and annual variations in food consumption and growth of a variety of fishes. These models are usually based on feeding and metabolic studies of age 1 and older fishes, and only a few studies provide data on young-of-the-year (YOY). What data are available suggest that weight-specific metabolic rates are very high for larval and juvenile fish (Mills and Forney 1981; Dabrowski et al. 1988) and that bioenergetic models developed from data on yearling and adult fish tend to underpredict food consumption and metabolic rates of YOY (Post 1990).

One important use of bioenergetic models is the prediction of prey consumption requirements in order to determine the carrying capacity of waterbodies for cultured or stocked fish (e.g. Stewart et al. 1981; Hurley 1986; Carline 1987). However, since many stocking programs involve the culture and transplantation of larval or juvenile fish, bioenergetic models developed from data on larger fish may not be useful for this purpose.

Another potential problem with the use of existing bioenergetic models to predict juvenile prey requirements is that they either ignore population density altogether or they model density only in terms of the amount of food available to individual fish (e.g. Cuenco et al. 1985). In a study of YOY walleye (*Stizostedion vitreum vitreum*) reared at three different densities in outdoor ponds, Fox and Flowers (1990) found that growth was density dependent and that prey consumption alone did not adequately explain this phenomenon. The authors hypothesized that density-dependent growth occurred partly because active metabolism increased with fish density. If this is true, bioenergetic models must account for these density-related metabolic effects in order to provide accurate predictions.

In this paper, I focus upon the utility of a commonly used bioenergetic model (Kitchell et al. 1977) for predicting the prey consumption rate of pond-reared YOY walleye. I use this model to test the following hypotheses: (1) juvenile walleye consumption and respiration rates are higher than those predicted from allometric models based on adult fish (Post 1990) and (2) growth efficiency and metabolism increase with population density.

Materials and Methods

Growth and Consumption Data

The study was conducted at the Whie Lake Fish Culture Station, 80 km north of Kingston, Ontario. Two-day-old

walleye were stocked at three densities (20, 40, and 60·m^{-3}) in eight fertilized rearing ponds. Fish were stocked in May 1988 and reared for 6 wk. Pond temperatures ranged from 14 to 25°C during this period. Walleye growth and stomach contents were monitored at 7-d intervals from samples of fish netted between 10:00 and 12:00.

Consumption rates based on diel sampling were determined for 17-d-old fish in a low- and a medium-density pond and 38-d-old fish from a different set of low- and medium-density ponds. Fish were netted at 3-h intervals over 24 h and preserved in 70% ethanol. Fresh dry weights of walleye were estimated from specimens previously preserved in ethanol and oven-dried for 24 h at 60°C. Stomach contents of 10 fish per sample were enumerated, measured, and converted into dry weight using a length–weight regression for chironomids in these ponds and published length–weight regressions for all other prey types (Mason 1977; Smock 1980; Culver et al. 1985).

Stomach evacuation rates were calculated from the maximal rate of decline in stomach content weight (grams per gram of body weight) in the midnight to dawn period when fish were not feeding. The maximal rate of evacuation on a given date was applied to both ponds, as temperatures and fish weights were not very different. Consumption for the 24-h period was estimated from the mean weight-specific stomach contents in each sampling period and the calculated exponential coefficient of stomach evacuation, using the method of Elliot and Persson (1978).

As a check on the above method, three stomach evacuation rate estimates were made on walleye harvested from the ponds. For the first estimate, 45-d-old (mean weight 0.75 g) walleye were held indoors in a 400-L trough at 23°C, starved for 12 h, and then fed to satiation at 07:00 with chironomid larvae collected from the pond. Ten fish were sacrificed when feeding ceased and at subsequent 1-h intervals. Only fish that had food in their guts were used in averaging hourly stomach contents. The exponential coefficient of evacuation was calculated using the Elliot and Persson (1978) equation.

The other two stomach evacuation estimates were made in 1990 using *Daphnia galeata* collected from the ponds as the test prey. For these estimates, I used 43-d-old walleye (mean weight 0.72 g) held at 21°C. In one trough, fish were fed *Daphnia* to satiation, and 10 individuals were sacrificed at hourly intervals. Experimental conditions in a second trough were identical to the first, except that 500 chironomid larvae 5–15 mm in length were added after the initial sacrifice of fish. The second experiment was performed to simulate continuous feeding. In this experiment, I calculated the rate of *Daphnia* evacuation using only those fish that consumed chironomids.

Finally, a larger set of daily consumption estimates was made for periods in which only late morning stomach contents were sampled, by using a fixed ratio of stomach content biomass to estimated daily consumption, generated from the feeding periodicity data. This ratio was fairly constant in the four ration estimates (mean ± SD 0.051 ± 0.011). Mean rate of consumption was estimated for 16-, 23-, 30-, and 37-d-old fish in each of the eight ponds. Six of the 32 weekly estimates gave extreme values (i.e. growth efficiencies >100%) and were therefore not used in my analysis. Five of these extreme estimates were generated on 16-d-old fish (mean weight <70 mg).

Bioenergetic Model

I used the Hewett and Johnson (1987) update of the Kitchell et al. (1977) bioenergetic model for walleye to simulate fish growth and consumption. This model is based upon the energetic equation

$$\text{Growth} = \text{Consumption} - (\text{Respiration} + \text{Egestion} + \text{Excretion}).$$

Consumption rate is calculated as a function of body weight, temperature, and prey availability. Respiration is modeled as a function of temperature, body weight, activity, and specific dynamic action; egestion and excretion are modeled as functions of consumption and temperature. Consumption parameters were derived from studies of Kelso (1972) and Swenson and Smith (1973) on age 2 and older walleye whereas respiration parameters were derived from a study by Solomon and Brafield (1972) on adult perch (Kitchell et al. 1977; Table 1). All model outputs are expressed in weight-specific terms (grams per gram of body weight per day). Parametric values used in the model were those given in Hewett and Johnson (1987) for walleye.

Comparison of Pond Data with Model Output

The bioenergetic model was used to generate two types of output: (1) predicted weight increases under maximal rates of prey consumption and (2) predicted rates of prey consumption under actual pond temperatures and walleye growth rates. Weight increase simulations were generated for a 35-d period. Temperatures were set at the mean weekly values of the eight ponds, and consumption was set at the maximum weight- and temperature-specific rate estimated by the model. Six simulations were run, varying the active to standard metabolic ratio (ACT) and the temperature of optimal consumption (CTO). ACT was set at either 1:1 (Kelso 1972; Minton and McLean 1982), 2:1 (Winberg 1956), or 3:1 (Ware 1975). A CTO of 22°C was used by Kitchell et al. (1977) from the data of Koenst and Smith (1976); however, earlier data by these authors (Smith and Koenst 1975, cited in Hokanson 1977) suggested a CTO of 25°C for smaller juveniles. To select ACT and CTO values, I assumed that the growth trajectory predicted with maximal temperature-related consumption rates should approximate that of walleye in the fastest growing ponds up to age 30 d, the point at which prey scarcity had obviously limited growth (Fox and Flowers 1990).

Weekly simulations were run on each pond with the selected form of the model. Predicted consumption rates were compared with the four diel sampling estimates and the 26 estimates extrapolated from late morning stomach contents.

Weight-specific metabolic outputs from this model were very high with the weights of walleye studied. I therefore compared outputs from this model with those generated by substituting weight-specific metabolic estimates made from two other models: (1) Mills and Forney (1981) for actively feeding YOY yellow perch and (2) Minton and McLean (1982) for sauger (*Stizostedion canadense*) (Table 1).

Effect of Walleye Density on Growth Efficiency and Metabolism

I tested for significant density effects on growth efficiency (weight gain per gross consumption) with the data set generated from late morning stomach contents. Since growth efficiency in walleye decreases with weight (Kelso 1972; this study), I used analysis of covariance (ANCOVA) to test the density hypothesis.

TABLE 1. Metabolic equations tested in the Kitchell et al. (1977) bioenergetic model for walleye. Definitions: SMR is standard metabolic rate, expressed in Eq. (1) as g·g wet weight^{-1} and in Eq. (3) as J·h^{-1}; M is total metabolic rate from SMR, activity, and specific dynamic action, measured at 20°C; T is temperature in °C; W is body weight, expressed as wet in Eq. (1) and (3) and dry in Eq. (2).

Equation	Source
(1) $\text{SMR} = (0.035 \cdot W^{-0.2}) \cdot (6.4 - 0.2T) \cdot e^{(-2.9 + 0.1074T)}$	Hewett and Johnson 1987
(2) $\ln M = 4.49 + 0.54 \cdot \ln W$	Mills and Forney 1981
(3) $\text{SMR} = 0.803 \cdot 10^{(0.037T)} \cdot W^{0.85}$	Minton and McLean 1982

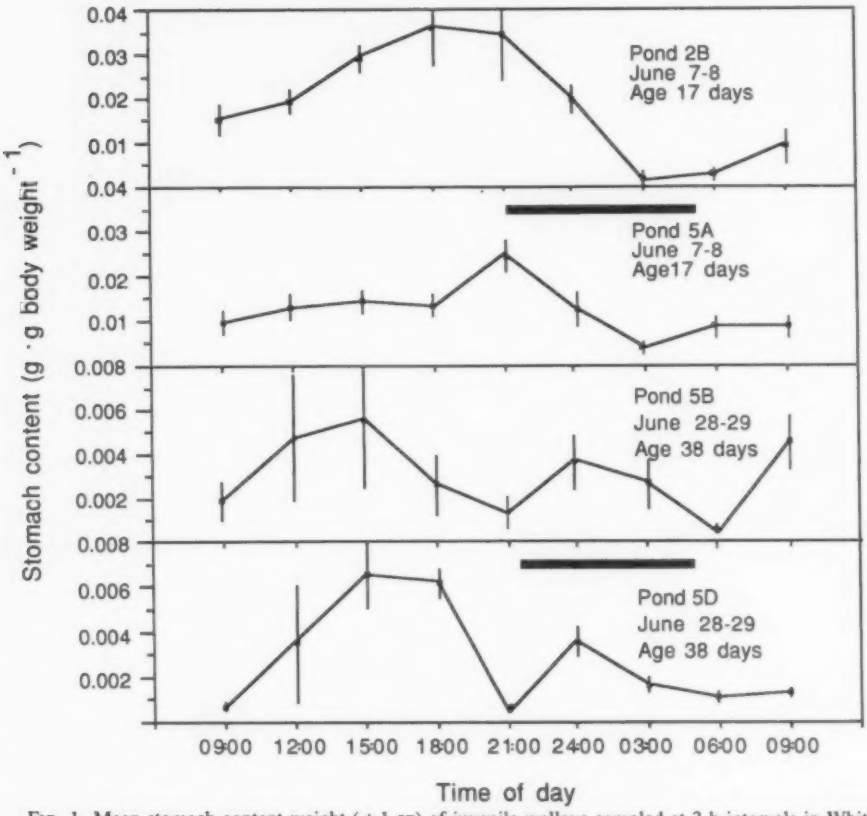

FIG. 1. Mean stomach content weight (± 1 SE) of juvenile walleye sampled at 3-h intervals in White Lake rearing ponds. Horizontal bars indicate period from dusk to dawn.

If metabolism increases with fish density, fish at higher densities require a relatively high ration to achieve a given level of growth, and the ratio of consumption predicted by the model to that estimated in situ should decrease with density. This hypothesis was tested with analysis of variance (ANOVA). The level for significance was set at $P = 0.05$ for both statistical tests.

Results

Juvenile Walleye Food Consumption

Mean daily ration estimates ranged from 4.6 to 39.2% of body weight in the four diel samples (Fig. 1; Table 2) and from 2.2 to 49.3% in the 26 late morning samples (Table 3). Specific consumption rates were generally lowest in the older fish, but the difference was strongly influenced by low availability of chironomids and *Daphnia* (their principal prey items) in the later weeks of the experiment (Table 2).

Diel ration estimates were based on instantaneous rates of evacuation of 0.85·h^{-1} for the 17-d-old walleye and 0.61·h^{-1} for the 38-d-old walleye. The comparable evacuation rates for 0.72- to 0.75-g walleye fed chironomids, *Daphnia* only, and *Daphnia* with continuously available chironomids were 0.31, 0.39, and 0.43·h^{-1}, respectively.

Growth Modeling

Predicted walleye weight at the maximal rate of food consumption was greatly influenced by ACT and CTO. Of the six ACT and CTO combinations examined, only one (ACT = 1, CTO = 22°C) matched the trajectory and exceeded the growth rate of walleye in the fastest growing pond populations (Fig. 2). Further testing of the model was conducted with these parameter values.

Modeling of Food Consumption

Predictions from the base model exceeded all four in situ consumption estimates made from diel sampling, with a mean

TABLE 2. Estimated growth and consumption rates of four juvenile walleye populations in rearing ponds, based on diel sampling of stomach contents.

Basic information				
Pond	Stocking density (m^{-3})[a]	Temp. (°C)	Age (d)	Mean wet wt (mg)
2B	40	19.5	17	81
5A	20	19.0	17	84
5B	40	21.8	38	548
5D	20	22.8	38	711

Calculated rates and prey data						
					Prey abundance[d]	
Pond	Evacuation rate (h^{-1})	Consumption (% body wt·d^{-1})	Growth[b] (% body wt·d^{-1})	Growth efficiency (%)	*Daphnia* (L^{-1})	Chironomids ($g \cdot m^{-2}$)
2B	0.85	39.2	18.2	46.4	5.5	9.1
5A	0.85	25.7	19.4	75.5	38.6	7.7
5B	0.61	4.9	0.4	8.2	0	0.5
5D	0.61	4.6	1.5	32.6	0	0.3

[a]Stocking density at the beginning of the experiment.
[b]Calculated from the change in mean weight of samples captured the day before and 6 d after feeding periodicity assessment. Age, temperature, and fish weight given are for the day of assessment.
[c]Ratio of specific growth rate to specific consumption rate.
[d]Estimated the day prior to feeding periodicity assessment using five Schlindler trap and five core samples.

TABLE 3. Estimated growth and consumption rates of juvenile walleye populations in rearing ponds, based on late morning stomach content analysis.[a]

Pond	Age (d)	Stocking density (m^{-3})	Temp. (°C)	Mean wet wt (mg)	Consumption (% body wt·d^{-1})	Growth (% body wt·d^{-1})	Growth efficiency (%)
2B	16	40	20.5	66	33.1	19.6	59.2
5C	16	60	18.0	51	21.4	16.0	74.8
5D	16	20	17.6	68	32.0	17.8	55.6
2A	23	60	21.2	148	21.5	14.7	68.3
2B	23	40	21.0	190	49.3	14.7	29.8
5A	23	20	20.8	184	31.5	16.4	52.1
5B	23	40	20.2	180	42.1	18.2	43.2
5C	23	60	21.2	130	24.8	23.0	92.7
5D	23	20	19.5	199	35.9	19.1	53.2
6	23	20	21.0	217	30.8	16.1	52.3
2A	30	60	24.0	318	13.5	−0.6	0
2B	30	40	24.0	414	16.5	−0.2	0
3	30	60	23.5	394	16.2	2.1	13.0
5A	30	20	23.0	455	15.8	7.1	44.9
5B	30	40	23.8	477	17.9	3.3	18.4
5C	30	60	23.2	432	35.6	1.7	4.8
5D	30	20	23.0	555	14.4	3.9	27.1
6	30	20	23.2	524	6.0	0.7	11.7
2A	37	60	19.5	314	10.1	3.7	36.6
2B	37	40	20.0	360	3.5	−1.6	0
3	37	60	18.5	469	5.8	2.8	48.3
5A	37	20	19.5	727	5.7	0.7	12.3
5B	37	40	20.0	614	2.9	0.4	13.8
5C	37	60	19.5	498	10.5	−0.3	0
5D	37	20	20.0	712	2.2	2.1	96.6
6	37	20	20.2	558	15.4	3.6	23.4

[a]All data, except for growth rate and stocking density, were estimated for, or recorded on, the day of stomach content analysis. Growth rate was calculated with the Kitchell et al. (1977) model for the day of stomach content analysis assuming a constant proportion of maximal weight and temperature-specific consumption.

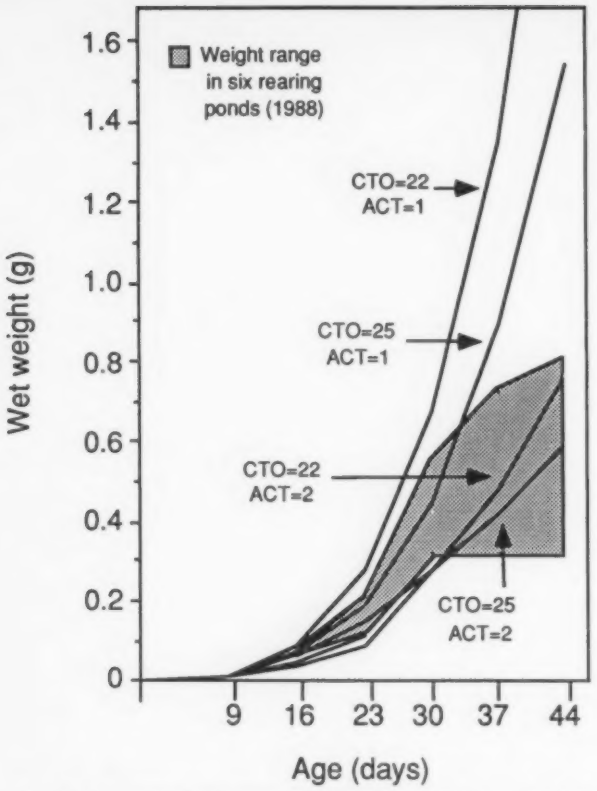

FIG. 2. Comparison of growth trajectories predicted from the bioenergetic model of Kitchell et al. (1977) and observed growth of pond walleye. The model was run with maximal temperature and weight-specific consumption rate and mean daily pond temperatures. Parameters varied were activity level (ACT) and temperature of optimal consumption (CTO). Model runs with ACT = 3 (not shown), are well below the growth trajectory range of pond walleye.

model to field ratio of 1.7 (Table 4). Substitution of the Minton and McLean (1982) respiration equation resulted in considerably lower consumption predictions. This occurred because Kitchell et al. (1977) standard metabolic rate estimates ranged from 4 to 4.6 times those obtained from the Minton and McLean (1982) equation on walleye ranging from 0.06 to 0.8 g wet weight. However, none of the model forms tested gave predictions consistently within 20% of consumption estimates (Table 4; Fig. 3).

All model predictions showed similar correlations with in situ consumption estimates ($0.75 < r < 0.77$). Once again, model to field estimates were highly variable in individual weeks and ponds, but the base model clearly overestimated average consumption (Table 5). Only the Minton and McLean (1982) 2·SMR model prediction average was close to that of in situ estimates.

An ideal fit between observed and predicted consumption values would show a regression slope of 1 and an intercept of 0. From this perspective, the Kitchell et al. (1977) model gave the least divergence from the ideal slope, and the Minton and McLean (1982) models gave the least divergence from the ideal intercept (Table 5). However, as shown in Fig. 4, the Minton and McLean 2·SMR model gave predictions that most closely matched consumption estimates over a broad range of consumption values.

Effect of Walleye Density on Growth Efficiency and Metabolism

Walleye weight was significant as a covariate in the analysis of fish density effects on walleye growth efficiency (efficiency $\log_e$ transformed: $F_{1,20} = 8.1$, $P = 0.01$). Regression slopes among the three densities were not significantly different in the covariate model ($F_{2,20} = 1.5$, $P = 0.24$). Mean growth efficiency was highest in low-density ponds (antilog of log-transformed values adjusted for the covariate: low = 44%, medium = 9%, high = 13%), but the three densities were not significantly different ($F_{2,22} = 3.0$, $P = 0.07$).

A second test to determine if fish density affected the metabolic expenditures of juveniles was made by comparing the model to field consumption ratios among the three density treatments. Fish at the lowest stocking density had the highest mean model to field consumption ratio, but again the difference among treatments was not significantly different (ANOVA with $\log_e$-transformed ratio: $F_{2,23} = 1.3$, $P = 0.28$).

Discussion

My results show that the Kitchell et al. (1977) bioenergetic model overestimates juvenile walleye consumption considerably, at least when used with suggested parameter values. Replacement of the standard metabolic equation with the more conservative equation of Minton and McLean (1982) gave predictions that were much closer to in situ estimates, particularly

TABLE 4. Comparison of in situ juvenile walleye consumption estimates with predicted output of Kitchell et al. (1977) model using original and two alternative respiration submodels.

		Consumption (% body wt·d^{-1})					
Pond	Age (d)	In situ estimate	Kitchell	M&F[a]	M&M[b]	2M&M[b]	3M&M[b]
2B	17	39.2	41.1	41.7	33.9	36.1	38.2
5A	17	25.7	42.8	42.2	35.7	37.6	39.6
5B	38	4.9	8.7	7.1	3.7	5.2	6.7
5D	38	4.6	11.2	8.1	5.7	7.1	8.6
Mean model to estimate ratio			1.73	1.48	1.06	1.25	1.44

[a]Mills and Forney (1981) equation for active feeding respiration of YOY *Perca flavescens*.
[b]Minton and McLean (1982) equation for standard metabolic rate of *Stizostedion canadense*, multiplied by 1–3 to estimate active metabolism.

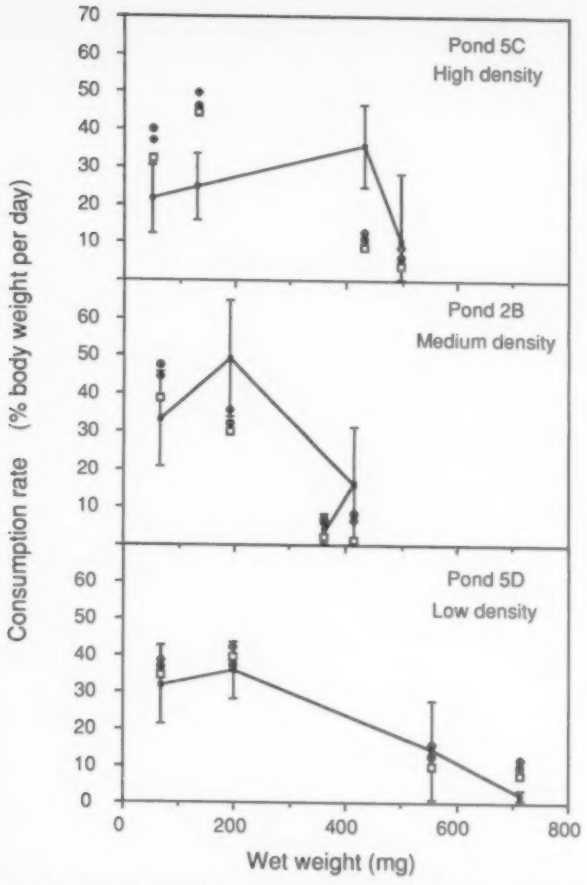

FIG. 3. Comparison of in situ consumption estimates in high-, medium-, and low-density ponds with consumption predicted by the Kitchell et al. (1977) base model (◇), the model with the Mills and Forney (1981) equation for active feeding respiration (⊙), and the model with the Minton and McLean (1982) equation for standard metabolic rate and an activity multiplier of 2 (□). Vertical bars represent 95% confidence intervals of estimates, based on a sample size of 10 fish.

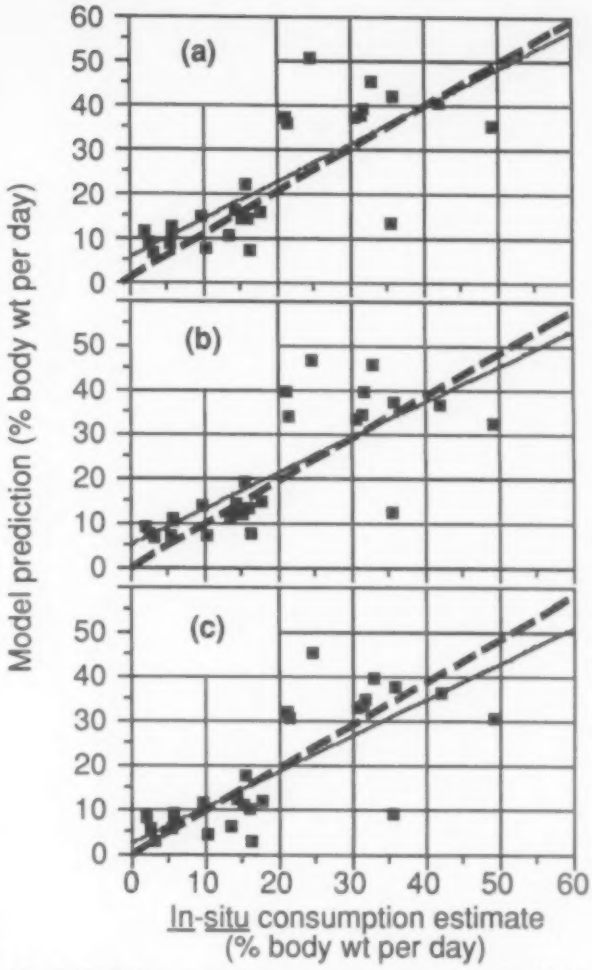

FIG. 4. Predictions versus in situ consumption estimates for (a) the Kitchell et al. (1977) bioenergetic model for walleye, (b) the model with the Mills and Forney (1981) metabolic equation, and (c) the model with the Minton and McLean (1982) standard metabolic rate with active to standard ratio of 2. Solid lines are fitted least squares regressions; broken lines show predictions equal to estimates.

TABLE 5. Regression of predicted consumption rates in bioenergetic models against in situ ration estimates(n = 26) based on late morning stomach contents of fish.

	Model[a]				
	Kitchell	M & F	M & M	2 M & M	3 M & M
	Regression ($y = a + bx$)				
a	0.057	0.051	0.006	0.023	0.039
b	0.848	0.805	0.802	0.812	0.822
r^2	0.58	0.57	0.58	0.58	0.59
P	<0.001	<0.001	<0.001	<0.001	<0.001
	Predicted to estimated consumption ratio (mean ± 95% CI)				
	1.42 ±0.37	1.29 ±0.31	0.88 ±0.25	1.05 ±0.27	1.22 ±0.31

[a]For an explanation of models tested see Table 4 and text.

when used with an ACT of 2. These data suggest that the allometric function for standard metabolism in the Kitchell et al. (1977) model is too steep (i.e. the rate of metabolic increase with the decrease in walleye weight is too high).

My results seemingly conflict with those of Post (1990), who showed that consumption and respiration rates of larval and juvenile perch are underestimated by adult allometry. However, the respiration equation in the Kitchell et al. (1977) model is based on yellow perch laboratory measurements, and this may be inappropriate for walleye. A comparison of my data with those of Mills and Forney (1981) and Post (1990) suggests that YOY perch have a higher weight-specific consumption rate than walleye. Since metabolism increases with food consumption, young yellow perch are likely to have a higher metabolism than young walleye. However, even if one assumes that the Minton and McLean (1982) metabolic model for sauger is appropriate for walleye, juvenile walleye consumption was

underpredicted only when an ACT of 1:1 was assumed (Table 5). This is unlikely to be the case for juvenile fish.

Assuming again that the Minton and McLean (1982) metabolic equation is appropriate, my results suggest that active metabolism in pond-reared YOY is about twice standard metabolism. This is lower than the ACT of 4.4 found in lake YOY perch by Post (1990), but slightly higher than the 1.6 calculated by Post (1990) from the data of Mills and Forney (1981) for juveniles fed in aquaria. Both Kelso (1972) and Minton and McLean (1982) suggest a 1:1 ratio for adult walleye in lakes. Thus, a 2:1 estimate is consistent with the assumptions that (1) juvenile fish are more active than adults (Dabrowski et al. 1988; Post 1990), (2) metabolism in a seminatural (pond culture) environment is higher than that in experimental chambers (see Winberg 1956), and (3) young walleye have a lower metabolic rate than young perch.

Model evaluations of this type depend upon the accuracy of gastric evacuation rates. Obtaining an appropriate gastric evacuation rate for field populations is difficult in food consumption studies because the rate of gastric evacuation can be affected by the number and size of prey eaten (Mills et al. 1984), stomach fullness (Noble 1973), and the continuity of feeding (Noble 1973). In my study, a 40% increase in the calculated evacuation rate would have resulted in a near 1:1 ratio of predicted consumption (Kitchell et al. model) to estimated in situ consumption. The probability of such an error in my estimates is low, given the much lower evacuation rate in my indoor experiments ($R = 0.31–0.43 \cdot h^{-1}$ versus 0.85 and $0.61 \cdot h^{-1}$ for smaller fish in situ). The time of complete stomach evacuation in my trough experiments was approximately 6 and 8 h with 45- to 48-mm walleye fed *Daphnia* and chironomids, respectively. These times are somewhat higher than the 7.25-h evacuation time found by Mathias and Li (1982) in 21-mm walleye fed *Daphnia*, but considerably lower than those reported for juvenile perch by Noble (1973) and Mills et al. (1984).

In my continuous feeding experiment, less than 25% of the fish fed to satiation ate subsequently over the 7-h period. This suggests that juvenile walleye are not continuous feeders in the manner of perch (Noble 1973) and gives further evidence of the major metabolic differences between the two percid species.

Although mean growth efficiency was highest, and active metabolism was lowest in walleye reared at the lowest density, my study was unable to show a significant density effect on metabolism. However, this lack of significance could have been due to high variability in the daily consumption rate of juvenile walleye. Daily consumption fluctuates in fish, and fluctuations around an average value may be considerable (Smagula and Adelman 1982; Tackett et al. 1988). With juvenile fish, irregular variations in "average" consumption imposed upon rapid change with age could result in consumption estimates on a given day which are unrepresentative of weekly trends. This variation could explain why six data points in my experiment gave growth efficiencies above 100%. A few unrepresentative data points would not greatly affect the average model to field ratio with 26 estimates. However, such data would have a much greater effect on statistical tests of comparison (such as ANCOVA and ANOVA) conducted with the same data set.

Another source of difficulty in demonstrating a population density – metabolism relationship is confounding by a food consumption – metabolism relationship. Active metabolism and the rate of food consumption are positively related in piscivores (Kerr 1982) and in age 1–3 yellow perch (Boisclair and Leggett 1989). Walleye stomach content data from my study indicate that relative rates of consumption fluctuated irregularly among ponds and density treatments (Table 3). This was partly due to fluctuations in prey density (Fox and Flowers 1990), which, in itself, would be expected to affect the energy expended to obtain food.

A population density effect on fish metabolism has yet to be demonstrated in the field. As shown here, this is a difficult task because of problems in estimating average consumption and in controlling extraneous variables (such as temperature and food supply) that affect the metabolic rate of fish.

Finally, the applicability of my pond results to more natural environments should be considered. Stocking densities in my study were two orders of magnitude higher than the maximum reported YOY density in lakes (reviewed in Colby et al. 1979), but mean walleye biomass at harvest ($19 \text{ g} \cdot \text{m}^{-2}$) was only 1.5–2 times that of littoral zone fish biomass in several mesotrophic lakes in Ontario and Quebec (Keast and Harker 1977; Hanson and Leggett 1985). Since pond fertilization results in much higher invertebrate prey biomass than that found in lakes (Fox et al. 1989), relative prey availability in the two types of systems may be comparable.

An assumption built into existing bioenergetic models is that biotic factors affect metabolism only by affecting the quantity of food obtained. If this assumption is valid, a model should work equally well in lakes and rearing ponds. The absence of a significant fish density effect on metabolism in my study gives support to the above assumption, but the trend in my data indicates that further testing for density-dependent metabolic effect is warranted.

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Effects of Moose Browsing on Decomposition Rates of Birch Leaf Litter in a Subarctic Stream

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Leaves from birch trees previously browsed by moose differed from leaves from unbrowsed trees in food quality for stream detritivores in an Alaskan subarctic stream. Leaves from previously browsed plants decomposed faster. Effects of browsing were tested by collecting leaves from previously browsed and unbrowsed trees and measuring loss of mass over time in an Alaskan subarctic stream. The browsing history of birch trees was associated with increased leaching rate of tannin, foliar nitrogen concentration, and rate of mass loss. All three factors were higher for leaves from trees previously browsed than for unbrowsed ones. Faster loss of tannin through leaching and higher foliar nitrogen concentration apparently caused birch detritus to be processed more rapidly by stream biota, potentially increasing secondary production of stream consumers. Hence, moose browsing was associated with important changes in the food quality of birch leaf litter, linking terrestrial herbivory and aquatic food webs.

Dans un cours d'eau subarctique de l'Alaska, les feuilles de bouleau auparavant broutées par des orignaux étaient différentes des feuilles d'arbres non broutées en terme de qualité de la nourriture pour les détritivores fluviaux. Les feuilles d'arbres auparavant broutées se décomposent plus rapidement. La quantification de la perte de masse de feuilles auparavant broutées et de feuilles non broutées en fonction du temps a permis de déterminer les incidences du broutage. Le broutage des bouleaux est associé à une augmentation du taux de perte de tanins, de la concentration de l'azote foliaire et du taux de perte de masse. Ces trois facteurs étaient nettement plus élevés dans le cas des feuilles de bouleau auparavant broutées. Un taux de perte plus élevé des tanins et une concentration plus élevée de l'azote foliaire semblent accroître le taux de transformation des feuilles par la biote fluviale, et donc la production secondaire potentielle des prédateurs fluviaux. Ainsi le broutage des feuilles par les originaux est lié à d'importants changements dans la qualité de la couche de feuilles de bouleau mortes et établit un lieu entre les herbivores terrestres et les réseaux alimentaires aquatiques.

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Herbivory can alter chemistry of woody plants and hence food value of uneaten leaves and leaves produced after browsing. In some situations, food value increases following herbivory (Danell et al. 1985; Bryant et al. 1991) and in others it decreases (Rhoades 1979; Haukioja and Neuvonen 1985; Haukioja 1980). In any case, leaves not eaten by herbivores ultimately abscise, become litter, and decompose. Because the decomposability or quality of litter is influenced by leaf chemistry, changes in leaf chemistry induced by herbivory have the potential to alter decomposition rates and, therefore, alter the transfer of energy and nutrients through and among ecosystems. One such transfer is from terrestrial to stream ecosystems via litter from riparian vegetation (Cummins et al. 1989). We report that quality of leaf litter from Alaska paper birch trees (*Betula resinifera* Britt. (Dugle 1966) = *B. papyrifera* var. *humulis* (Reg.) Fern. & Raup (Hulten 1968)) browsed by moose (*Alces alces* L.) differs from litter from unbrowsed birches, thereby affecting rate of litter processing by stream biota. Furthermore, we propose that browsing-mediated changes in foliar tannin structure or foliar nitrogen concentration (or both) may cause an increase in decomposition rate of this litter.

Small stream ecosystems in forested regions to a large extent depend on energy input from abscised leaves of riparian trees (Minshall 1978; Cummins and Klug 1979; Vannote et al. 1980). After entering the stream, these leaves undergo a rapid (approximately 1–2 d) loss of mass due to leaching followed by fungal and bacterial colonization. After microbial conditioning, leaves become palatable to macroinvertebrates, and processing rates increase because shredders ingest large pieces of plant material (Anderson and Sedell 1979; Webster and Benfield 1986). If the quality of leaf litter is altered as a consequence of browsing, stream secondary production might be either enhanced or reduced by terrestrial herbivore activity (Irons et al. 1988; Stout 1989).

Methods

We tested effects of moose browsing on decomposition rates in Monument Creek, a subarctic headwater stream near Fairbanks, Alaska (65°N latitude, 146°W longitude). We used leaves from paper birch, a boreal tree common in riparian habitats of interior Alaska. Senescent leaves were collected from branches that regrew from recent browsing and from trees lacking browse damage. We estimated that browsing had occurred 1–2 yr previous to sampling. Browsed trees were not noticeably affected by other herbivory, for example, recent snowshoe hare browse or insect defoliation. Trees were approximately 2.5 cm in diameter (breast high), and at least 20 browsed and 20 unbrowsed trees were sampled. Leaves from trees within a

treatment were composited to reduce the effect of between-tree variance. Twenty-five leaf packs (3 g dry mass) from browsed and unbrowsed trees were placed in 2-cm-mesh net bags in the stream, and five packs for each treatment were removed on days 2, 14, 28, 56, and 75. These packs were dried at 55°C and weighed and then combusted at 550°C and reweighed for ash-free dry weight (AFDW) determination. We also analyzed these samples for condensed tannin and nitrogen. Tannins were assayed by the butanol–HCl reaction for proanthocyanidin: 75 mg of freeze-dried leaf material was extracted twice sequentially in 20 mL of aqueous methanol (50% vol/vol) at 95°C, and values are expressed as quebracho tannin equivalents (Bryant et al. 1985). Nitrogen analysis was performed on a Technicon autoanalyzer using sulfuric/selenious acid digestion and colorimetric analysis with a ferricyanide blue reaction for nitrogen (Chapin and Kedrowski 1983).

Results

Leaves from trees browsed by moose were processed (decomposed) significantly faster than leaves from unbrowsed trees (Fig. 1) (treatment effect (T): $p = 0.001$, date × treatment interaction ($D \times T$): $p = 0.001$, two-way ANOVA with date and treatment as independent variables). Unleached leaves from browsed and unbrowsed trees possessed similar initial concentrations of tannin. However, tannin in leaves from browsed birch trees disappeared more rapidly than from leaves of unbrowsed trees (T: $p = 0.001$, $D \times T$: $p = 0.001$; Fig. 1). Nitrogen was higher in leaves from browsed trees than in those from unbrowsed trees both in unleached and leached samples (T: $p = 0.006$, $D \times T$: $p = 0.020$; Fig. 1).

Discussion

Browsing by moose was associated with qualitative changes in birch foliar tannin, which were reflected in the different rates of tannin loss or leachability. This differs from the quantitative changes in levels of secondary compounds (e.g. tannin) predicted by existing plant defense models, e.g. induced defense (Haukioja 1980) and carbon/nutrient balance (Bryant et al. 1983). These changes in condensed tannin leaching rates mediated by browsing may be related to differences in their degree of polymerization or the frequency at which they are covalently bound with cell wall material (Haslam 1989). Structural analysis (nuclear magnetic resonance) of tannin suggests that the latter hypothesis is the most likely (T. P. Clausen, Department of Chemistry, University of Alaska, Fairbanks, AK, pers. comm.). Browsing was also associated with increased leaf nitrogen concentrations, which were maintained through the decomposition process.

The increased rate of mass loss in litter from browsed trees could be caused by a decreased deterrent or toxic effect of tannin or by an increased concentration of nitrogen as protein, or both, on microbial or invertebrate utilization of leaves (Zucker 1983). Use of plants naturally browsed by herbivores in tests between browsed and unbrowsed vegetation requires the assumption that herbivores browsed these plants randomly with respect to the variables measured (Strauss 1988; Neuvonen and Haukioja 1985). The alternative hypothesis is that the moose detect preexisting differences in the foliage and browse nonrandomly. That is, is the patchiness (Pickett and White 1985) in leaf litter food quality caused by moose, or do moose perceive an existing patchiness? In either case, litter quality is different.

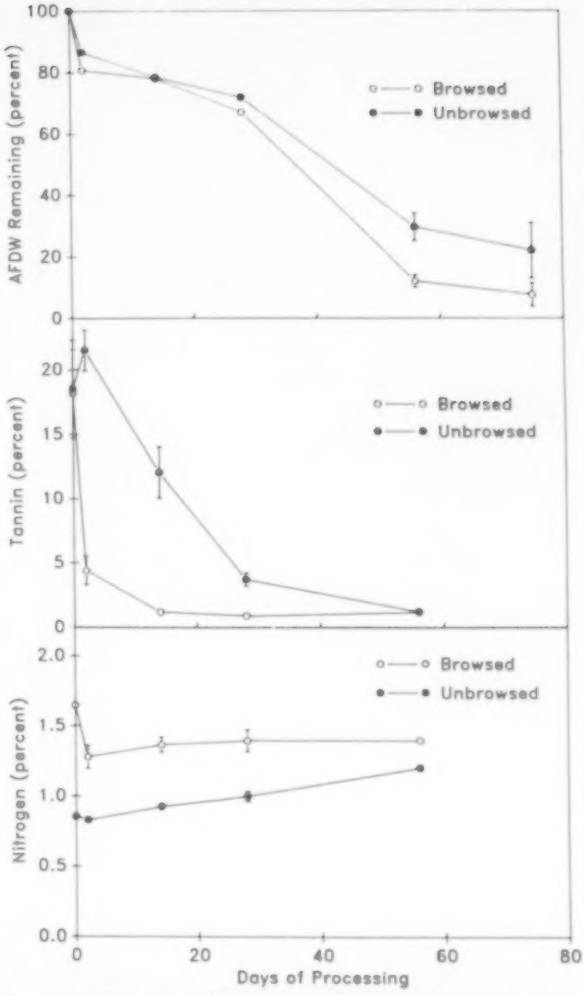

FIG. 1. Decomposition of birch leaves in Monument Creek. Top: Mass loss (AFDW = ash-free dry weight). Error bars are 1SE of the mean, N = 5, except day 75 ($n = 3$). Unapparent error bars are hidden by symbols. Middle: Tannin concentration (percent dry mass). Error and n are the same as in the top panel except day 56 leaves from browsed trees ($n = 1$). Missing data points were due to insufficient leaf material remaining for analyses. Bottom: Nitrogen concentration (percent dry mass). Error bars and n are the same as in the middle panel.

Moose have been implicated in long-term (approximately 40 yr) changes in boreal forest ecosystems. On Isle Royale, moose herbivory altered plant species composition, quantity and composition of litterfall, depth of forest floor, and soil chemistry and microbial biomass. Changes in soil characteristics were primarily related to replacement of deciduous species, producing high-quality leaf litter, by coniferous ones producing recalcitrant needle litter (Pastor et al. 1988). By potentially altering the biochemistry of deciduous leaves, we suggest that moose may have short-term impacts on stream ecosystem food webs 1–2 yr after browsing. Perturbations to terrestrial ecosystem processes for many temporal and spatial scales may have profound effects on stream communities, for

example, elimination of beaver (Naiman et al. 1988) and American chestnut (Smock and MacGregor 1988) from much of their former ranges. Because many aquatic insects depend on leaves from riparian trees for food, increased food quality should translate into faster insect growth, enhancing secondary production. Thus, the food webs of streams in forested regions are tightly coupled with the riparian forest. Short-term and long-term ecological changes in the terrestrial ecosystem may have effects that ripple through the aquatic ecosystem, affecting production of microorganisms, aquatic macroinvertebrates, and higher order consumers such as fish. This experiment provides evidence that herbivory on riparian vegetation affects aquatic food webs.

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Effects of Environmental pH on the Hepatic Mixed Function Oxidases in Atlantic Salmon (*Salmo salar*)

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Atlantic salmon (*Salmo salar*) were held for some months in the Medway River, N.S., at pH 5.2–5.5 and in the Westfield River, N.S., in either untreated ("acidic") water at pH 4.7–5.2 or limed water at pH 51.–5.9 and were examined for effects on hepatic monooxygenases. Fish from the Westfield acidic regime had lower body weights than those from the other two regimes, but similar liver weights. Females generally had higher hepatic microsomal protein contents than males, but this variable was not affected by environmental pH. Westfield acidic fish usually had higher benzo[a]pyrene hydroxylase and lower ethoxyresorufin *O*-deethylase activity than those from the other two regimes. Cytochromes P450 and b_5 showed no clear difference between groups. Although the different pH environments affected reproductive success and steroid hormone metabolism, such changes were not reflected in these components of the hepatic monooxygenase system.

Les saumons de l'Atlantique (*Salmo salar*) ont été gardés pendant quelques mois dans la Medway River (N.-É.) à un pH de 5,2 à 5,5 et dans la Westfield River (N.-É.), soit dans de l'eau non traitée ("acide") à un pH de 4,7 à 5,2, soit dans de l'eau chaulée à un pH de 5,1 à 5,9, puis ils ont été examinés quant aux effets sur les monooxygénases hépatiques. Les poissons de la Westfield au régime acide avaient un poids inférieur à celui des poissons des deux autres régimes, mais le poids du foie était le même. Les femelles avaient généralement une teneur en protéines microsomales hépatiques plus forte que les mâles, mais cette variable n'était pas touchée par le pH de l'environnement. Les poissons en milieu acide de la Westfield River avaient généralement une activité d'hydroxylase benzo[a]pyrène supérieure et une activité d'éthoxyrésorufine-*O* des éthylases inférieure à celle des poissons vivant dans les deux autres milieux. Les cytochromes P450 et b_5 ne montraient aucune différence nette entre les groupes. Bien que les milieux de pH différents aient eu des effets sur le succès de la reproduction et sur le métabolisme de l'hormone stéroïde, les changements notés ne se répercutaient pas sur les composantes du système de monooxygénase hépatique.

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Fish hepatic monooxygenases are induced by a range of organic chemicals, which includes coplanar polychlorinated biphenyls, polynuclear aromatic hydrocarbons, and some chlorinated dibenzofurans and dibenzodioxins. Increases in the activity of these enzymes are usually interpreted as indicating the presence or effects of such chemicals. However, changes in hepatic monooxygenase activity in fish may also be correlated with age, sex, maturity, and treatment with some sex hormones and their derivatives (Stegeman and Chevion 1980; Förlin 1980; Vodicnik and Lech 1983). Indeed, some components of the monooxygenase system are involved in normal steroid hormone metabolism.

Changes in environmental pH resulting from acid precipitation may cause changes in the normal steroid hormone metabolism in salmonids (Freeman and Sangalang 1985; Sangalang et al. 1990). Such changes may, in turn, be associated with changes in hepatic monooxygenase systems. In this paper, we describe the results of studies to examine the possibility that fish maintained in environments of differing pH have different hepatic monooxygenase activity.

Materials and Methods

Sangalang et al. (1990) have described in detail the system in which fish were exposed to varying environmental pH. Briefly, 2-yr-old hatchery-reared Atlantic salmon (*Salmo salar*) were held in net cages in the Medway River, N.S., from spring until late summer 1986. They were fed 6.2-mm commercial trout grower pellets daily and the pH and temperature of the water were monitored daily. In mid-September 1986, approximately 40 fish (equal numbers of males and females of approximately the same body condition) were transferred from the

Medway River to two holding tanks which were supplied with water from the Westfield River, N.S., either untreated or treated with crushed limestone. The fish continued to be fed daily, and pH and temperature of the water in the tanks were monitored.

In early December 1986, 12 fish from each of the three regimes ("Medway," "Westfield acidic," and "Westfield limed") were anesthetized by immersion in a 1 : 20 000 dilution of buffered MS-222 in river water. There were six males and six females in each group except for the Westfield acidic group where there were seven females and five males. Total body weight and sex were recorded. Livers were removed and weighed, and an accurately weighed sample of approximately 1 g was homogenized in 3 mL of 1.15% KCl with five or six strokes in a Teflon–glass Potter–Elvehjem homogenizer. The homogenate was centrifuged at 10 000 × *g* for 15 min at ambient temperature (0°C). The resulting supernatant was stored as a glycerol solution on dry ice (Tamburini et al. 1984) for transport to the laboratory. There, microsomes were prepared by diluting the thawed supernatant 1:10 with 1.15% KCl and centrifuging at 100 000 × *g* for 40 min. The microsomal pellet was resuspended in a suitable volume of 1.15% KCl.

Benzo[*a*]pyrene hydroxylase (BPH) was assayed using the method of Nebert and Gelboin (1968). Cytochromes P450 and b_5 were assayed in the microsomal pellet as described by Edwards et al. (1988). Ethoxyresorufin *O*-deethylase (EROD) was determined (Burke and Mayer 1974) in 0.1 M phosphate buffer, pH 7.5, at 20°C using excitation and emission wavelengths of 510 and 585 nm and with the substrate concentration 1.92 μM.

Benzo[*a*]pyrene and NADPH were obtained from Sigma Chemical Co. (St. Louis, MO) and 3-hydroxybenzo[*a*]pryene from Midwest Research Institute (Kansas City, MO). Resorufin was purchased from Pierce Chemical Co. (Rockford, IL) and ethoxyresorufin was synthesized using the procedure of Burke and Mayer (1974).

Proteins were determined by the method of Lowry et al. (1951) using bovine serum albumin as standard. Data were analysed statistically with the BMDP package (Dixon et al. 1983).

Results

During the exposure period, the pH of the Medway River ranged from 5.2 to 5.5 (pH 5.2 on the sampling date). Natural water (Westfield acidic) from the Westfield River ranged from pH 5.0 to 4.7 (pH 4.7 on the sampling date) and limed water from the Westfield was slightly higher than the untreated water (pH 5.3 on the sampling date). More detailed analyses of the variation in pH are given in Sangalang et al. (1990).

Body weights of Medway and Westfield limed fish did not differ significantly (either grouped or separated by sex); Westfield acidic fish were smaller than the other groups, but the difference was statistically significant only between males (Table 1). Within exposures, male and female body weights did not differ. Liver weights did not differ between exposures, but females had larger livers than males (significant only in the Medway group). Liver expressed as a fraction of total body weight (LSI) was higher in the Westfield acidic group (reflecting lower body weight) but this was significant only in males (Table 1). Microsomal protein content did not differ within sex and between exposures, but females always had higher protein contents than males (Table 1).

Indices of monooxygenase activity showed no clear patterns. Within sex and between exposures, the Westfield acidic males had somewhat higher BPH activity than the other groups. However, male EROD activity was lower in the Westfield acidic group (although it did not differ significantly between any of the exposures). In females, BPH did not differ between groups, but EROD was (like the males) lower in both Westfield groups than in the Medway group (Table 1). Some of the differences between sexes were exaggerated when expressed as activity per gram of liver fresh tissue, due to the consistently different liver protein contents between sexes. Other components of the monooxygenase system (cytochromes P450 and b_5) showed no consistent pattern of variation with exposure (Table 1).

Discussion

Hepatic monooxygenase activities have apparently not been reported in *Salmo salar*, but activities found in the fish from these exposures were generally within the same range as those described for other salmonids. Thus in *Oncorhynchus mykiss* (formerly *S. gairdneri*) (which has been used extensively for experimental studies of monooxygenase induction), EROD activities in uninduced fish are typically less than 100 pmol·mg protein^{-1}·min^{-1} (Eisele et al. 1984; Addison et al. 1987) and BPH activities are usually in the range of 1–10 nmol·mg protein^{-1}·h^{-1} (e.g. Voss et al. 1982; Stegeman 1981).

None of the three environmental regimes caused any large or consistent differences in hepatic monooxygenase activity. There was a slight general increase in BPH levels (significant only in males) and a slight general decrease in EROD activities (significant only in females) in the series Medway, Westfield limed, and Westfield acidic. These trends are difficult to interpret, however, since the changes are both inconsistent and small. The different responses of EROD and BPH are difficult to explain: EROD and BPH are probably catalysed by the same P450 isozyme (Stegeman and Kloepper-Sams 1987), and thus, activities of the two enzymes would be expected to be positively correlated. This has observed in some studies: in winter flounder (*Pseudopleuronectes americanus*) sampled at intervals over a period of 2 yr, EROD and BPH activity were well correlated ($r = 0.476$, $n = 164$, $P < 0.01$ in males; $r = 0.661$, $n = 172$, $P < 0.01$ in females) (Edwards et al. 1988). In the present study, EROD and BPH were not correlated ($P > 0.1$ in both males and females). The absence of any significant correlation probably arises from various factors which could include the small numbers of samples and low enzyme activity (contributing to larger errors in activity estimations).

Experimental induction of monooxygenases in *O. mykiss* may lead to EROD and BPH increases anywhere from 2- to 100-fold (e.g. Voss et al. 1982; Addison et al. 1987). In field studies of monooxygenase induction in another salmonid (*Salvelinus namaycush*) by adventitious exposure to pollutants, both enzymes were increased by about sixfold (Binder and Lech 1984), and similar changes have been seen in other nonsalmonid species (e.g. Spies et al. 1982; Payne et al. 1984; Addison and Edwards 1988).

In the present study, differences in environmental pH regimes caused changes in reproductive success in salmon (which may reflect changes in the timing of steroid hormone production) and in kidney cortisol metabolism (Sangalang et al. 1990). EROD and BPH vary with sexual maturity and reproductive status (Stegeman and Chevion 1980; Förlin 1980), and other components of the hepatic monooxygenase system respond to changing plasma cortisol levels (Hansson and Lidman 1978). However, the poor correlation between EROD and BPH activ-

TABLE 1. Biological variables and indices of hepatic monooxygenase activity in salmon (*Salmo salar*) exposed to different pH environments as described in the text (data as mean ± SD). *Within each sex*, data in the same row followed by a different letter are significantly different ($P < 0.05$).

Variable	Males			Females		
	Medway (n = 6)	Westfield limed (n = 6)	Westfield acidic (n = 5)	Medway (n = 6)	Westfield limed (n = 6)	Westfield acidic (n = 7)
Fish wt. (g)	981 ± 124 a	974 ± 39 a	782 ± 125 b	993 ± 192 a	898 ± 135 a	766 ± 87 a
Liver % fish wt. (LSI)	0.9 ± 0.1 a	1.0 ± 0.1 a	1.2 ± 0.1 b	1.4 ± 0.1 a	1.2 ± 0.2 a	1.4 ± 0.2 a
Microsomal protein (mg·g liver^{-1})	14.5 ± 3.2 a	14.7 ± 3.5 a	16.7 ± 2.0 a	23.8 ± 3.5 a	18.8 ± 2.9 a	21.1 ± 2.7 a
BPH (nmol·mg protein^{-1}·h^{-1})[a]	1.57 ± 0.90 a	2.25 ± 0.83 ab	3.43 ± 1.46 b	2.25 ± 0.92 a	3.64 ± 1.72 a	2.52 ± 1.55 a
EROD (pmol·mg protein^{-1}·min^{-1})[b]	33.5 ± 11.0 a	22.3 ± 13.4 a	18.3 ± 14.4 a	34.9 ± 18.0 a	16.3 ± 13.1 ab	11.0 ± 8.5 b
Cytochrome P450 (nmol·mg protein^{-1})	0.02[c] a	0.06 ± 0. 01 a	0.06 ± 0.03 a	0.05 ± 0.01 a	0.05 ± 0.03 a	0.04 ± 0.01 a
Cytochrome b_5 (nmol·mg protein^{-1})	0.03 ± 0.01 a	0.03 ± 0.01 a	0.05 ± 0.01 a	0.02 ± 0.01 a	0.03 ± 0. 01 a	0.04 ± 0.03 a

[a]Benzo[*a*]pyrene hydroxylase.
[b]Ethoxyresorufin *O*-deethylase.
[c]One sample only analysed.

ities and the relatively small changes in them observed in this work suggest that they are not appreciably affected by environmental pH. It is now clear, however, that the monooxygenases involved in steroid hormone metabolism usually involve P450s different from those indicated by BPH and EROD activity (e.g. Nebert et al. 1987), and these isozymes may respond to changes in environmental pH.

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Herring Recruitment in Maine Coastal Waters: An Ecological Model

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Campbell, D. E., and J. J. Graham. 1991. Herring recruitment in Maine coastal waters: an ecological model. Can. J. Fish. Aquat. Sci. 48: 448–471.

An ecological model of larval herring survival in Maine coastal waters demonstrated, quantitatively, that herring recruitment is the result of a complex interaction of many processes, no one of which is truly dominant. Herring larval abundance measured on a series of spring cruises from 1965 to 1973 was used to estimate survival. Two-year-old recruitment to the Maine sardine fishery was established in the larval stage in some years, but not until the brit stage in other years. A sensitivity analysis of the model showed that food supply in winter and autumn and the quantity and distribution of spawning were primary factors controlling recruitment in years when the larval stage was critical. Density-dependent predation on brit by opportunistic pelagic and demersal predators was proposed as a mechanism determining recruitment in years of high larval herring survival. In the model, the Maine coast was divided into four spatial sectors of different area and characteristics. The western coastal waters from the headlands to the 100-m isobath made the largest contribution to recruitment in most years. Maine herring management regulations that limit fishing on spawning aggregations of herring should increase larval herring survival along the Maine coast in most years.

Un modèle d'ordre écologique de la survie des larves de hareng dans les eaux côtières du Maine a révélé que le recrutement du hareng est le résultat d'une interaction complexe de nombreux processus dont aucun n'est vraiment prépondérant. Le taux de survie a été déterminé à partir de l'abondance des larves de hareng quantifiée au cours d'une série de croisières effectuées au printemps de 1965 à 1973. Certaines années, le recrutement de poissons de deux ans à la pêche de la sardine du Canada dans les eaux du Maine s'est effectué au stade larvaire, mais d'autres années, seulement au stade post-larvaire. Une analyse de sensibilité du modèle a révélé que la quantité de proies disponibles en hiver et en automne, ainsi que le nombre et la répartition des géniteurs étaient les principaux facteurs contrôlant le recrutement pendant les années où le stade larvaire était critique. La prédation liée à la densité que les prédateurs pélagiques et démersaux opportunistes exercent sur les post-larves est avancée comme le mécanisme qui fixe le recrutement pendant les années où le taux de survie des larves de hareng est élevé. Dans le modèle, la côte du Maine a été divisée en quatre secteurs spatiaux à superficie et caractéristiques différentes. Les eaux côtières occidentales s'étendant du promontoire à l'isobathe de 100 m contribuent le plus fortement au recrutement pendant la plupart des années. Les règlements de gestion de la pêche du hareng dans les eaux du Maine visant à limiter la pêche dans les frayères du hareng devraient amener une augmentation du taux de survie des larves dans les eaux côtières du Maine pendant la plupart des années.

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Recruitment has always been one of the most difficult terms to estimate in the stock equation of Russell (1931). Although fisheries scientists have often used the simplified relationships developed by Ricker (1954) and Beverton and Holt (1957) to describe the influx of new recruits to a population, there is seldom a good fit between the data and theory (Hall 1988). Because of this it is generally recognized that recruitment is a complex ecological process which is determined by the interrelationship of many biological and environmental variables. One way to explore the recruitment process more fully is with mathematical models. Ecological models of varying complexity can be used to study the recruitment process in well-studied fisheries, as well as poorly known fisheries.

Long-term research on the early life history stages of herring from the Maine coast has established sufficient information on recruitment processes to permit the development of detailed models. Graham (1982) proposed a conceptual model of herring larval recruitment in Maine coastal waters which separates inshore larval mortality into two phases, a period of density-dependent mortality in the autumn and a density-independent or environmentally related mortality associated with the overwintering period. In Graham's conceptual model, larval survival is enhanced by late spawning which shortens the overwintering period and by greater dispersal along the coast which decreases intraspecific competition for food resources by opening a larger support area to the larvae. In this view the estuarine and nearshore waters shallower than 100 m are a nursery area for larval herring which for a given year establishes a carrying capacity for them on the Maine coast. Year-to-year variations in the number and distribution of larvae spawned, the amount of zooplankton food available to them, and the environmental conditions to which they are subjected will determine the extent to which this potential productive capacity is utilized. In this study we build on the concepts of Graham (1982) to develop a mathematical model that can explain year-to-year variation in the springtime larval herring abundance in Maine coastal waters for the period from 1965 to 1973. We use the model to evaluate the relative importance of the external forcing functions such as food, spawning, temperature, longshore transport, and pre-

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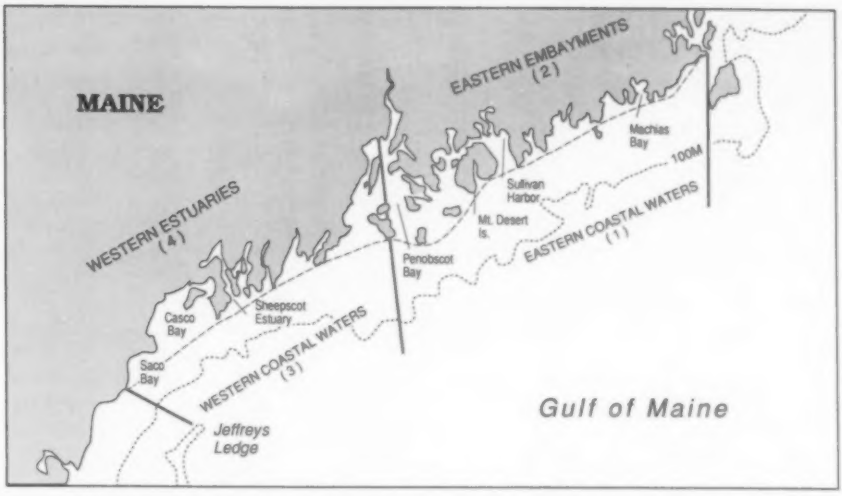


FIG. 1. Maine coast showing the spatial boundaries of the four model sectors numbered 1 through 4. Estuarine ecosystems are separated from the coastal ecosystems by the broken line along the headlands. The seaward boundary of the coastal sectors is the 100 m isobath.

dation in determining the number of herring larvae that survive to metamorphosis. In addition, we relate the estimates of springtime larval survival predicted by the model to the recruitment of 2-yr-old herring along the Maine coast.

Background Information

Most of the information on larval abundance for the period from 1965 to 1973 comes from the Sheepscot Estuary and the central coastal section of Graham (1982). The boundaries of his central coastal section are about the same as the western coastal sector (Fig. 1). Fall and winter measurements of herring larvae in the Sheepscot estuary (Graham 1982) are assumed to represent the western estuarine area in the model. Larval herring biomass was estimated from the available spring cruise data (Graham 1982) and used as the output function for our model.

Figure 1 divides the Maine coast into eastern and western halves by drawing a line that passes through Vinalhaven and Matinicus islands. The position of this boundary was based on evidence that the climatological and oceanographic processes governing marine ecosystem functions are of different intensity along the eastern and western portions of the Maine coast (Bigelow 1927; Gran and Braarud 1935; Sherman 1976; Townsend et al. 1986). Solar radiation is usually greater in the western section than in the east primarily due to more frequent cloud cover in the eastern sector (Gran and Braarud 1935). Bigelow (1927) and Yentsch and Garfield (1981) showed greater vertical mixing along the eastern part of the Maine coast. Garrett et al. (1978) related the extent of mixing throughout the Gulf of Maine to differences in tidal mixing. The average tidal height in the eastern coastal sector is about 25% greater than in the western coastal sector. Differences in vertical mixing along the Maine coast may in part be due to wind (Yentsch and Garfield 1981; Loder and Greenberg 1986). Saunders (1977) showed that the average wind stress along the eastern Maine coast is about twice that found along the western coast in most seasons. The character of waters along the eastern Maine coast is modified by advection of high-nutrient, upwelled water from the mouth of the Bay of Fundy (Apollonio and Applin 1972; Brooks 1985; Townsend et al. 1987).

A brief summary of the ecology and life history of herring larvae in Maine coastal waters follows. Eggs are deposited on the bottom in both the eastern and western sections of the Maine coast (Boyar et al. 1973; Stevenson and Knowles 1988). Egg production is due primarily to fish age 4 and greater, although some herring recruit to the spawning population at age 3 (Anthony and Waring 1980). Spawning usually begins during August in eastern Maine and in late September in western Maine (Graham et al. 1972b), although spawning may occur as late as December (Graham 1982). After hatching the larvae accumulate in the vicinity of egg beds for a few days. Recently hatched larvae are subject to severe mortality as they concentrate on the spawning ground and attempt their first feeding (Graham and Chenoweth 1973). The breakup of this concentration and the transport of larvae southwestward coincides with an improvement in their nutritional condition and survival (Townsend et al. 1986). Sherman (1970) showed that macrozooplankton concentrations are generally several times higher along the western Maine coast than along the eastern coast. In the estuaries and embayments the accumulation of larvae leads to a severe density-dependent mortality (Graham 1982) followed by a less severe, but longer winter mortality. Comparison of the growth rate and mortality of several larval cohorts indicated that the high mortality suffered by relatively small larvae is due in part to predation as well as food limitation (Graham and Townsend 1985). Metamorphosis to the juvenile form occurs in the spring and the juveniles grow through the summer reaching a size sufficient for canning by the sardine industry during autumn. However, recruitment occurs primarily in the subsequent summer when the juveniles are at a most appropriate size for canning.

Model Development

The larval herring recruitment model is spatially divided into four cells numbered 1 through 4 (Fig. 1). Physical transport processes move larvae alongshore through cells 1 and 3, as well

as shoreward from coastal to estuarine waters (cell pairs 1,2 and 3,4). The lines drawn perpendicular to the shore form the eastern and western boundaries of the coastal water cells. The eastern boundary was set to include the known spawning areas along the eastern Maine coast and southwest of Grand Manan. The western boundary is positioned to include the coastal area usually influenced by larval transport from the east and to minimize input from Jeffreys Ledge spawning.

Energy circuit models (Odum 1971, 1983) describe the process of larval herring recruitment in Maine coastal waters (Fig. 2; Table 1); Campbell and Graham (1986) described an early version of this model. The ecosystem components are the same within each cell and consist of storages representing phytoplankton biomass (P), zooplankton biomass (Z), small herring larvae 7–15 mm in size (S), and large herring larvae (L) which are $>$ 15 mm in size. Only small herring larvae are subject to physical transport processes. We assumed that the development of the caudal fin at about 15 mm (Blaxter 1962) would give larvae greater than this size more control over their movements making them less susceptible to passive physical transport. There is evidence that larvae smaller than this size are able to adjust their height in the water column and thus they may have more control over their movements than in this model (Blaxter and Hunter 1982; Stephenson and Power 1988). Longshore transport of phytoplankton and zooplankton from impoverished eastern waters to the richer waters in the west was not included in our analysis.

In the model, phytoplankton production is controlled by solar insolation alone. This is not unreasonable for the light-limited waters of the eastern coastal section (Gran and Braarud 1935; Campbell and Wroblewski 1986) and may also be appropriate for the western section as well during most of the fall and winter. The zooplankton component includes both macro- and microzooplankton which feed on the phytoplankton and in turn are eaten by small and large herring larvae and other predators. Small herring larvae feed on the microzooplankton only (Sherman and Honey 1971) whereas large larvae utilize the entire zooplankton biomass. Small larvae develop into large larvae and both sizes of larvae are eaten by predators. In the coastal waters, small larvae are input to the model upon leaving the yolk sac stage (J_{Yi}). Spawning is assumed to have a normal distribution with the mean at September 25 and standard deviation of 16 d in the eastern sectors. In the west, spawning is centered on October 9 with standard deviation of 11 d (Graham et al. 1973). Temperature was considered to be an important variable controlling the growth and mortality of large larvae within the estuaries (Graham and Davis 1971; Anthony and Fogarty 1985). Larvae that remain in the coastal waters were not considered to be vulnerable to the effects of winter water temperature because it was reasoned that they could always find less extreme temperatures by seeking deeper water. Both temperature (J_{Temp}) and solar insolation (J_{Ii}) are entered into the model by linear interpolation between monthly average values.

Figure 2a shows that advective transport through the coastal cells is driven by the counterclockwise current in the Gulf of Maine (J_C) and by northeast winds (J_{NE}) which augment the coastal current transport (Day 1958; Greenberg 1983). The water which enters the eastern coastal section around Grand Manan carries few if any herring larvae with it (Chenoweth et al. 1989). Larvae are transported between the eastern and western sections, but some water and larvae move offshore at the Penobscot Bay. Further down the coast, water and a few larvae move southwestward out of the western section toward Massachusetts Bay.

Tidal dispersion acts to move small herring larvae shoreward under the assumption that differential advection in the coastal boundary current effectively forms an impenetrable seaward boundary whereas the estuaries and embayments act as an absorbing boundary. Fletcher (1985) has demonstrated through a random walk analysis of fish movements along a barrier screen that boundary conditions similar to those given above lead to a greater probability of fish moving toward the absorbing boundary. Thus, the physical factors which control water movements in the coastal waters of Maine supply boundary conditions which result in the inshore movement of larvae from chance alone. Wind-driven transport will also have a shoreward bias given these boundary conditions. Tidal tansport (J_T) is entered as a constant for each section of the coast, while wind-driven transport (J_W) is entered as a linear interpolation between the monthly mean values.

The Model as a Heuristic Tool

Initial simulations of the model in Campbell and Graham (1986) did not produce the detail observed in the spatial and temporal distributions of larval herring along the Maine coast. Therefore, we decided to use the model to learn about the processes controlling the spatial and temporal distribution of herring larvae. Through an iterative process between the modeler and the field scientist, we made a set of intuitive additions to the model based on assumptions backed up by field evidence which produced a plausible estimate of the spatial and temporal variability of herring larvae along the Maine coast. This revised model (see Fig. 2) incorporates several working hypotheses that may provide insights into the herring recruitment process.

The most noticeable feature of the 1971 larval herring biomass data was the rapid autumn increase followed by a precipitous decline (Fig. 3). The magnitude and steepness of this pulse was more pronounced in the western sector of the coast than in the east. Graham (1982) attributed this pattern to strong density-dependent mortality that was probably food related. With this starting point we assumed that the feeding relationship between herring larvae and their zooplankton prey was controlled by the ratio of larval herring biomass to zooplankton biomass as shown in Fig. 4d. We discovered that it was necessary to weight the zooplankton biomass using two factors which indicated its suitability as food for larval herring: zooplankton factor A, ZFA, determined the peak larval biomass and zooplankton factor B, ZFB, established the biomass of larval herring which survived following the fall die-off. These two factors determined the timing of changes in large larval mortality in the model, and by varying them in simulations we were able to accurately reproduce the shape of the fall die-off curve. There remained the task of linking these observations to the real world.

Sherman and Honey (1971) showed that the size of the zooplankton prey taken by herring larvae increased from autumn to winter (Fig. 5). The average length of herring larvae found in the western areas in mid-December is 22–24 mm which is just large enough to allow feeding on small copepods such as *Pseudocalanus* and *Oithona* that are 0.7–1.0 mm in length (Blaxter 1965). There is also a shift in the type of zooplankton eaten by larval herring in autumn compared with winter (Table 2). Larval herring fed mainly on copepodites and nauplii in the autumn whereas in winter their diet was dominated by

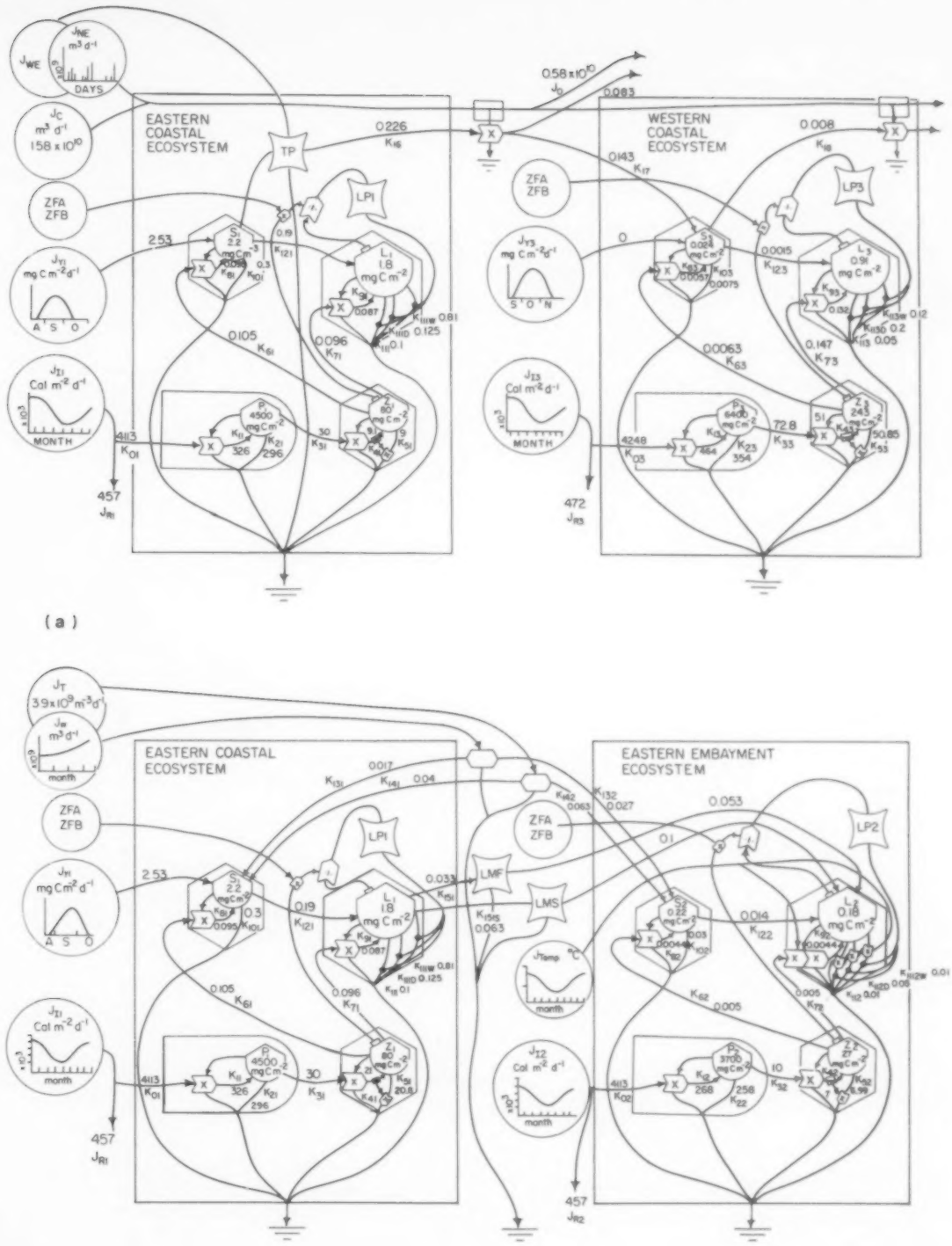
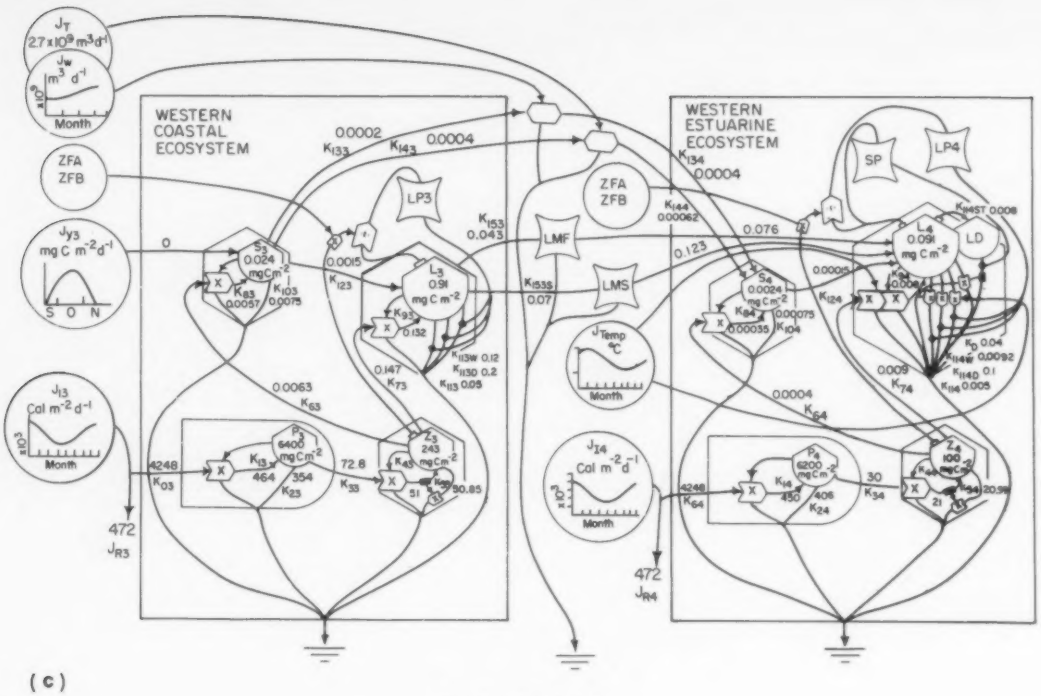


FIG. 2. Evaluated energy circuit models for pairs of sectors in Fig. 1 showing the transport mechanisms that link them. (a) The eastern and western coastal sectors (1 and 3) are linked by longshore transport; (b) the eastern coastal and embayment sectors (1 and 2) are linked through wind and tidal transport and larval movements; (c) the western coastal and estuarine sectors (3 and 4) are also linked through wind and tidal transport and larval movements. *(Fig. 2 concluded next page)*



(c)

FIG. 2. (*Concluded*)

TABLE 1. Equations for the model shown in Fig. 2. Some equations are slightly different in each spatial sector (1–4 in Fig. 1) designated by *i*.

(1) $J_{Ri} = J_{Ii}/(1+k_{1i}P_i)$; $J_{I1} = J_{I2}$, $J_{I3} = J_{I4}$

(2) $dP_i/dt = k_{1i}P_iJ_{Ri} - k_{2i}P_i - k_{3i}P_iZ_i$

(3) $dZ_i/dt = k_{4i}P_iZ_i(1 - k_{5i}Z_i) - k_{6i}Z_iS_i - k_{7i}Z_iL_i$

(4) $dS_i/dt = J_{Yi} + k_{8i}Z_iS_i - k_{10i}S_i - k_{12i}S_i$; $J_{Y2,4} = 0$
$- k_{13i}J_W[(S_{1,3}/60) - (S_{2,4}/40)]$
$- k_{14i}J_{Ti}[(S_{1,3}/60) - (S_{2,4}/40)]$
$- k_{16}TS_1\,(J_C + J_{NE})$; for S_1
$+ k_{17}TS_1(J_C + J_{NE})$; for S_3

(5) $dL_i/dt = k_{12i}S_i + k_{8i}Z_iL_i$
$- k_{11i}L_i$; until A true
$- k_{11ij}L_iJ_{Temp}$; for L_2 and L_4, $j = D, W$
$- k_{11iD}L_i$; A true if $L_i/Z_i(ZFA_i) > ZA_i$ until B
$- k_{11iW}L_i$; B true if A and $L_i/Z_i(ZFB_i) < ZB_i$
$- k_{151}L_{1,3} + k_{151}L_{2,4}$; if $T < (F - TD_1)$
$- k_{1515}L_{1,3} + k_{1515}L_{2,4}$; if $T > SP$
$- k_DLD$; for L_4 and $T = TS + TD_2$

where $dLD/dt = k_{1145T}L_4$; if $L_4 > ST$ and $T > TS$ until $TD_2 = 7$
$- k_DLD$ if $TD_2 > 7$

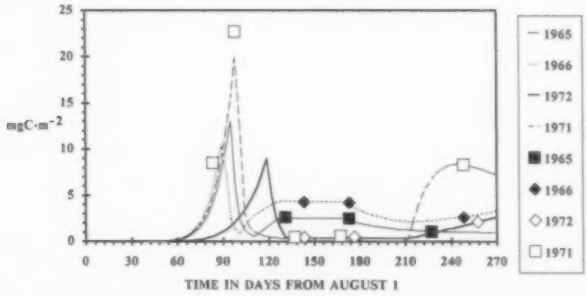


FIG. 3. Fall die-off pattern is demonstrated by model results (lines) and data (symbols) for four years. The fall die-off in the western estuaries was followed by higher survival during the 1960's than in the 1970's.

small copepod adults. Using this information we hypothesized that the peak in fall larval herring abundance was controlled by the quantity of zooplankton nauplii and copepodites available as food for herring larvae in a given year (ZFA in the model). We also hypothesized that the number of herring larvae surviving after the fall die-off was determined by the populations of small zooplankton adults, specifically *Pseudocalanus* and *Oithona*, represented by ZFB in the model.

Zooplankton factors A and B could be adjusted to give an accurate estimate of the herring larval biomass which survived to the spring in the eastern and western sectors, but the distribution of this biomass between the coastal and estuarine sectors could not be accurately represented. We assumed that the springtime spatial distribution of larvae was caused by the migration of herring larvae from coastal to inshore waters. In the model we simplified the spring migration by assuming that inshore movement began on March 1 of each year and could

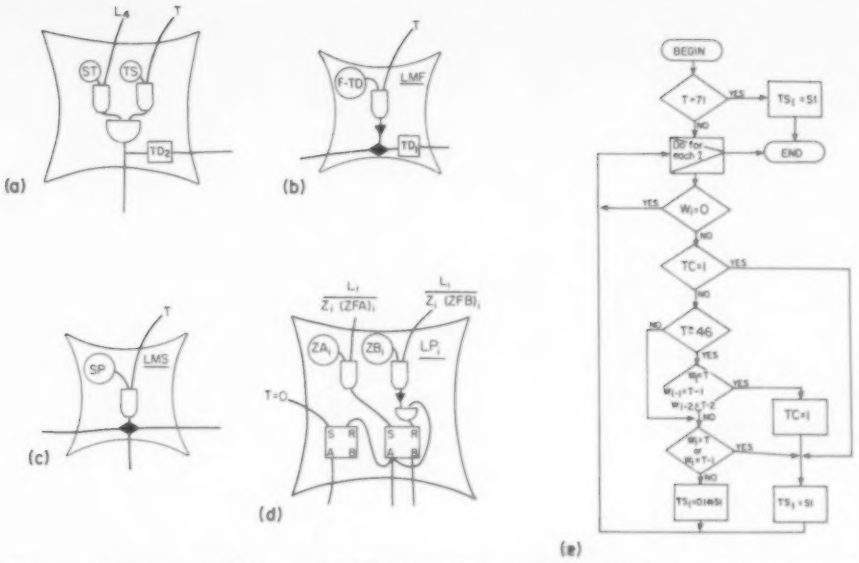


FIG. 4. Logic programs added to the model which simulate important processes that were not recognized initially. (a) Starvation program for the western estuaries; (b) fall inshore movement of large larvae; (c) spring inshore movement of larvae; (d) limits imposed by the autumn and winter zooplankton food of herring larvae; (e) wind events controlling the release of larvae from the eastern spawning area. A detailed description of these programs may be found in the Appendix.

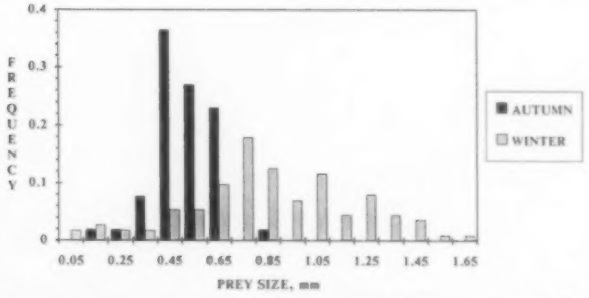


FIG. 5. Switch in the prey size of herring larvae from autumn to winter (adapted from Sherman and Honey 1971).

TABLE 2. Changes in the diet of larval herring from autumn to winter in Maine coastal waters (abstracted from Sherman and Honey 1971).

Food item category	Autumn (n = 55) (% feeding)	Winter (n = 73) (% feeding)
Copepod naupli	27.0	6.0
Copepodites	95.4	10.0
Pseudocalanus minutus	—	60.0
Oithona similus	4.5	31.4
Harpacticoids	4.5	1.4
Acartia sp.	2.3	5.7
Other species	—	8.6
Copepod remains	6.8	14.3

be of variable intensity from one year to the next. Thus, the inshore–offshore distribution of a given quantity of larval herring was entirely a function of the spring larval migration from coastal to estuarine waters (Fig. 4c).

Tidal transport of small larvae into inshore waters in the fall was not sufficient to supply the quantities of larval herring biomass measured there. Therefore, we assumed that large (>15 mm) herring larvae migrated from coastal to inshore waters in the fall in proportion to the amount of larvae in the coastal waters (Fig. 4b). Graham et al. (1972a) presented evidence which confirms that both small and large larvae contribute to the peak of abundance in western estuaries. In addition, simulations showing the timing of peak inshore biomass demonstrated that large larvae moved into the estuaries more slowly than small larvae which were transported by tidal dispersion. We incorporated a 2-wk delay between the time larvae appeared in the coastal waters and the time they arrived in the estuaries to account for the observed timing of the fall peak in herring larval concentrations in the Sheepscot Estuary.

Larval herring abundance in the Sheepscot Estuary during the period 1965–73 exhibited a complex pattern of survival (Fig. 3) which was difficult to explain. From 1965 to 1968 a large number of larvae survived the fall die-off, and the winter mortality rate calculated from 1971 data modified by water temperature was not sufficient to reduce the large number of larvae in early winter to the numbers found in the inshore waters during the spring. An additional mortality must have operated in the western estuaries during the 1960's. This mortality could be related to food, predation, or some other factor not considered in the model. Because abundance in the inshore waters after the fall die-off was far greater than the winter carrying capacity (see SP logic program in the Appendix), starvation could be an important source of wintertime mortality in the Sheepscot estuary and thus, we included this in our model (Fig. 4a). The starvation program turns on a flow which marks a quantity of larvae for starvation (LD in Fig. 2c). The quantity of larvae that starve depends on the amount of larval biomass present in a given year. Blaxter (1965) showed that 5–7 d without food push herring larvae past a "point of no return" after which starving larvae are incapable of feeding. Herring larvae in the western estuaries begin to die after passing a 7-d "point

of no return," and they continue to die at a rate that caused complete mortality within 30 d in 1965. Larvae lost from the LD storage are simultaneously removed from the L_4 storage.

We proposed several new mechanisms controlling longshore transport to estimate the significance of this factor in determining larval survival. Past research provided two clues that helped in formulating these mechanisms (Fig. 4e). First, Graham and Townsend (1985) noted that the transport time of different larval cohorts from the spawning ground to Sullivan Harbor was highly variable, ranging from 5 to 30 d for the years 1980–82. Second, Chenoweth et al. (1989) observed that larvae spawned at the same time and place had different movement histories. They observed that some larvae remained on the eastern Maine spawning grounds, while others were transported southwestward along the coast. Any physical mechanism explaining larval transport must account for the observed variable rates of cohort movement and the splitting of cohorts into stationary and moving components.

Watson's (1936) work provides the basis for a hypothesis which might explain larval herring movements along the Maine coast. Watson (1936) found that the homogeneity produced by tidal mixing in the waters from Grand Manan Channel to Petit Manan excludes permanent residual currents if enough light water is available over the shoal area to produce an outward gravity force sufficient to balance the inward dynamic force of the peripheral longshore current. Lighter water is maintained in this area from spring to fall first by the spring freshet and later in the summer by the greater warming of well-mixed shoal waters. Water on the shoal is less dense than water at a similar depth in the stratified peripheral zone, where density is largely determined by a steeper vertical temperature gradient. Watson (1936) pointed out that variable wind currents will occur more easily in homogeneous water than elsewhere, since there are no gravity forces to oppose them. We hypothesize that herring larvae spawned on the eastern Maine grounds may be released from their retention area by wind-driven currents which set the homogeneous water in motion. Larvae would also be released from this region if the supply of light water on the shoals were to be sufficiently eroded to allow the peripheral current to move shoreward. If wind events of sufficient magnitude move water and larvae away from the retention area and tidal mixing reestablishes the retention zone soon after the winds stop, we have a mechanism which can explain the patterns of larval movement observed by Chenoweth et al. (1989) and Graham and Townsend (1985).

In the transport program (Fig. 4e), larvae were released from the spawning areas at 1/10 the maximum rate in the absence of wind events due to the inherent leakiness of such circulation systems (Butman et al. 1982). After day 71 we assumed that fall storms had resulted in enough overturning and cooling to decrease the offshore density gradient to the point where lighter water no longer existed on the shoals in sufficient quantities to prevent the longshore current from transporting newly spawned larvae out of the retention area (Apollonio and Applin 1972). Release of larvae from the retention area from August 1 to October 10 was associated with wind events of 5 $m \cdot s^{-1}$ and greater. We assumed that each wind event began moving water instantaneously because both horizontal and vertical density gradients were small in the spawning area. Also, we assumed that tidal mixing would reestablish a homogeneous water column within four tidal mixing periods after the wind stopped; thus each day with average wind velocity greater than 5 $m \cdot s^{-1}$ resulted in approximately two days when larvae were allowed

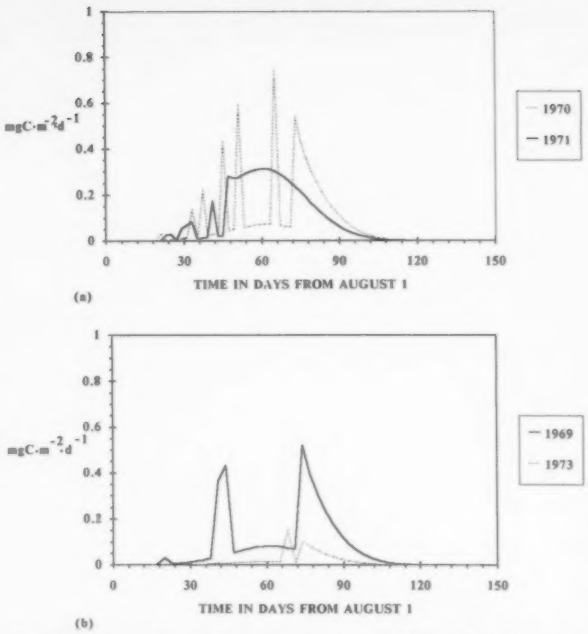


FIG. 6. Patterns of longshore transport in (a) 1970 when wind events were frequent and 1971 when a large storm occurred in mid-September and (b) 1969 when spawning was large and wind events infrequent and 1973 when spawning was small and wind events infrequent.

to travel at the maximum rate down the coast. We assumed that winds from any direction would result in the release of larvae from the retention areas given that small larvae were homogeneously distributed in the water column and that waters at depth would move in a different direction than the surface waters. Larvae driven offshore are effectively moved along the shore by the peripheral current, since this boundary could not be penetrated in the model nor did it appear to be breached in any significant way in the field data of Graham et al. (1972a, 1972b, 1973), Townsend et al. (1986), and Chenoweth et al. (1989). Large storm events which blew with an average wind avelocity of 5 $m \cdot s^{-1}$ or greater for three or more consecutive days and occurred after September 15 caused sufficient horizontal and vertical mixing of waters to preclude the reestablishment of the necessary density gradients after the event.

Four patterns of larval herring transport from eastern to western coastal waters could be discerned on the basis of the physical conditions discussed above (Fig. 6). In 1970, transport was dominated by intermittent wind events whereas in 1971, a long period of stormy weather resulted in early breakdown of the retention area (Fig. 6a). In 1969 and 1973 there were few wind events, but spawning was greater in 1969. The wind-driven release of herring larvae from their retention area was temporally variable from one year to the next, producing a diverse spatial distribution of larvae along the Maine coast which is similar in general pattern to that observed by Graham et al. (1972b, 1973), Townsend et al. (1986), and Chenoweth et al. (1989).

Simulation Methods

The additions and adjustments to the model described above were necessary to produce a good fit to the large larval biomass

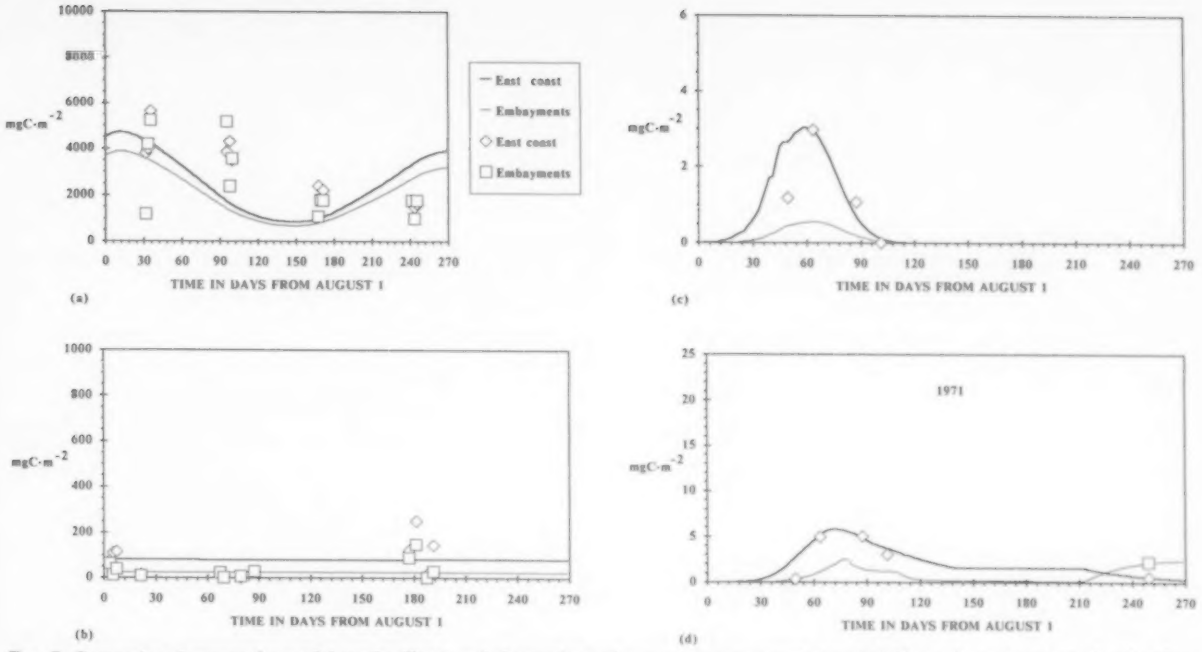


FIG. 7. Comparison between the model results (lines) and observations of ecosystem components (symbols) from the eastern coastal and embayment sectors in 1971. (a) Phytoplankton; (b) zooplankton; (c) small herring larvae; (d) large herring larvae.

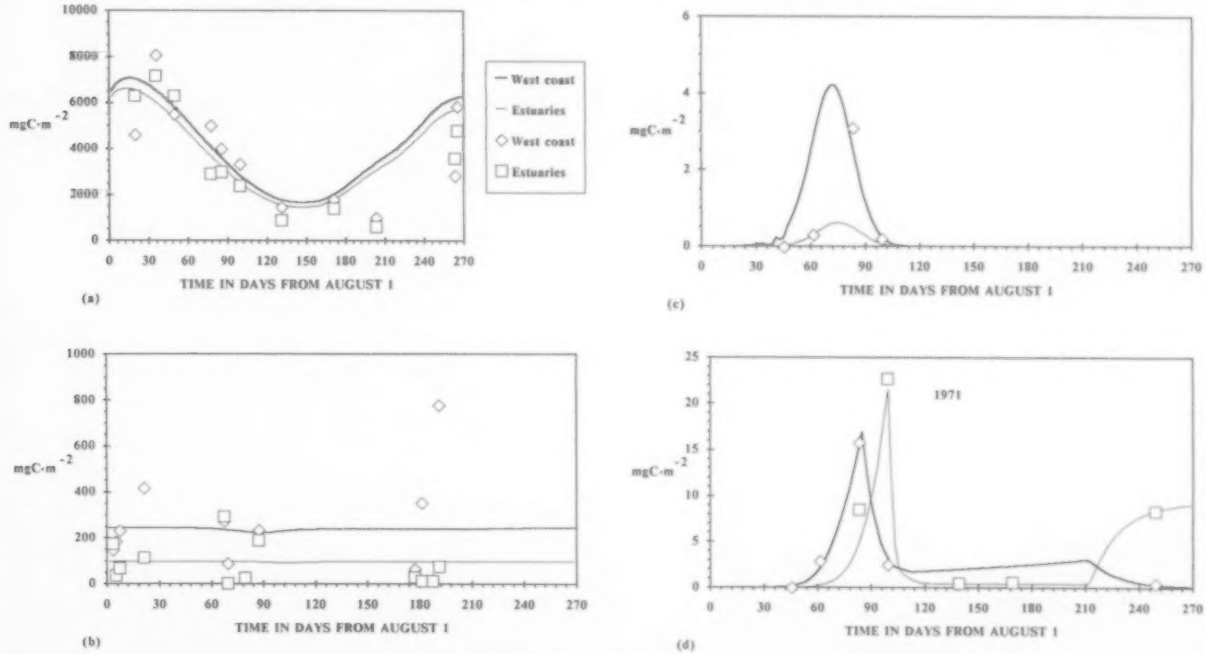


FIG. 8. Comparison between the model results (lines) and observations of ecosystem components (symbols) from the western coastal and estuarine sectors in 1971. (a) Phytoplankton; (b) zooplankton; (c) small herring larvae; (d) large herring larvae.

measurements made in 1971. Simulation methods used in fitting the model to the measurements of large larval biomass are explained below. First, a migration of large larvae into the estuaries was added and a delay estimated to reproduce the magnitude and timing of the larval herring biomass peak in the western estuaries. A similar delayed migration was applied to the eastern sectors based on our caclulations in the west. Next the density-dependent mortality coefficients, the k_{11iD}'s in the Appendix, were estimated to approximate the decline of large larval biomass in the 1971 data. Thresholds ZA_i and ZB_i (Fig. 4d) were set so that the high density-dependent mortality began and ended in each sector close to the levels of larval

biomass observed in 1971. The winter mortality in each sector was then set so that the total biomass which survived in both the coastal and estuarine sectors in the west was approximately equal to the total biomass estimated from the 1971 spring larval cruise. For the eastern sectors, no data existed on larval survival in the spring of 1971. We assumed that larval herring survival in the east during 1971 was similar to the average survival in the eastern sectors from 1973 to 1978, and winter mortality coefficients there were set based on this assumption. At this point the model was able to predict the larval herring biomass which survived in the eastern and western sections of the Maine coast in 1971, but it could not predict the distribution of that biomass between coastal and estuarine waters at the time of the spring cruise. A variable inshore migration factor was used in the west to fit the known inshore– offshore distribution of larval biomass in the spring. In the eastern area an inshore migration rate was assumed to reproduce the spatial distribution obtained from the 1973–78 average survival.

Simulation Results

Phytoplankton biomass curves from model simulation compared well with biomass estimates obtained from different locations in the western sectors (Fig. 8a). However, model estimates of phytoplankton biomass in the eastern sectors were somewhat too low in winter and too high in the spring (Fig. 7a). Simulation of zooplankton biomass showed a fairly constant population differing in magnitude between the east and west with average values that were reasonable first-order estimates of the observed zooplankton biomass (Fig. 7b and 8b). The model simulation results for small larvae (Fig. 7c and 8c) were fit to 1971 field measurements by adjusting the timing of spawning and the production of yolk sac larvae. The timing and distribution of spawning was not well known; thus it was reasonable to slightly modify this forcing function to fit the 1971 data. The maximum possible yolk sac larval production (see the Appendix) was required to produce the small larval biomass observed in the east (Fig. 7c), but only 80% of the maximum production of yolk sac larvae was necessary to match the observed biomass in the west (Fig. 8c). Using the logic programs described above, we obtained a very good fit between the model predictions of large larval biomass and the measured values in both the western sectors of the model and the eastern coastal sector (Fig. 7d and 8d).

Next the larval herring model was tested by simulating large larval survival using an independent data set from 1972 (Fig. 9). Only data from 1971, 1965, and 1973 were used in determining the structure of the model and the coefficient values. The time history of all forcing functions in 1972 was known except for the zooplankton population parameters (ZFA and ZFB) which were varied to give a good fit of the western sector model results to the data (Fig. 9b). The model parameters calculated for 1971 did not give a good fit to the east coast data, but if the autumn natural mortality of the large larvae, k_{111}, was decreased by 50%, an excellent fit was obtained (Fig. 9a). This was the only coefficient that had to be changed to fit our model to the data from the six years that we used to test it.

The model was used to simulate larval herring survival in 1969, 1970, and 1973, for which good fits of the model to the data (not shown) were obtained by changing only the zooplankton population characteristics. All other forcing functions were known except the actual spawning activity. For these years the maximum possible spawning activity, as calculated from virtual population analysis (see the Appendix), was used in the simulations. Only in 1973 could an estimate of springtime inshore migration be made for the eastern sectors. The total quantity of large larval biomass present in the east and west on March 1 was determined by the model; however, the final inshore–offshore distribution of these larvae is assumed to conform to field observations and was not predicted by the model. Spring inshore movement for the eastern area in all years, except 1971, was assumed constant and equal to the rate observed in 1973.

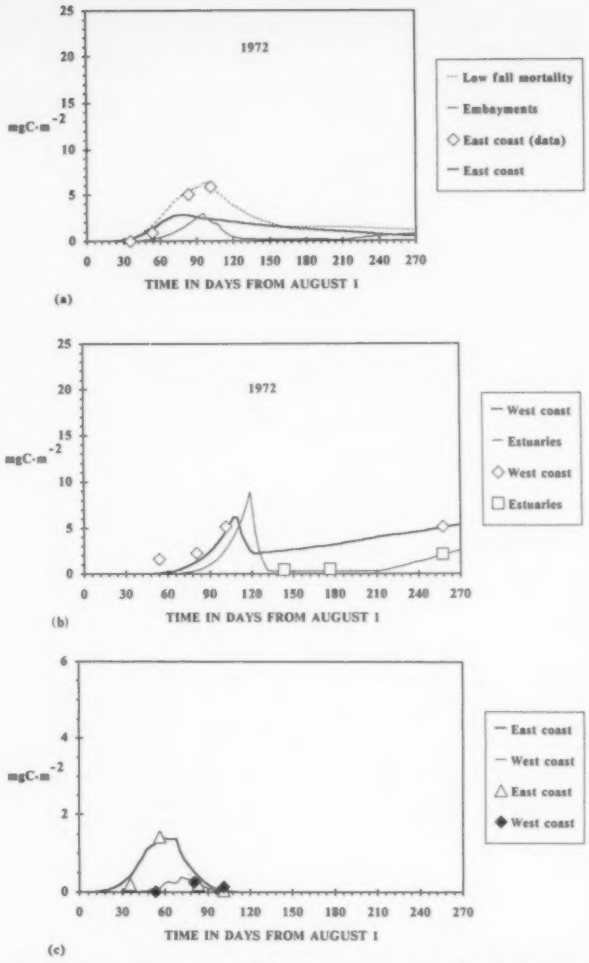


FIG. 9. Comparison of model simulation results (lines) with the 1972 data (symbols) using 1972 forcing functions but allowing ZFA and ZFB to vary. (a) Large larval biomass in the eastern coastal and embayment sectors obtained using 1971 coefficients; model results fit the data when the autumn mortality coefficient was decreased by 50%; (b) model predictions of large larval biomass in the western coastal and estuarine sectors compared with 1972 data; (c) small larval biomass in the eastern and western coastal sectors.

Zooplankton measurements were made along the Maine coast from 1964 through 1968 (Sherman 1966, 1968, 1970) which allowed us to estimate relative changes in the zooplankton population parameters, ZFA and ZFB, for these years. These zooplankton measurements covered the eastern and western coastal sectors but not the estuaries and embayments. This information

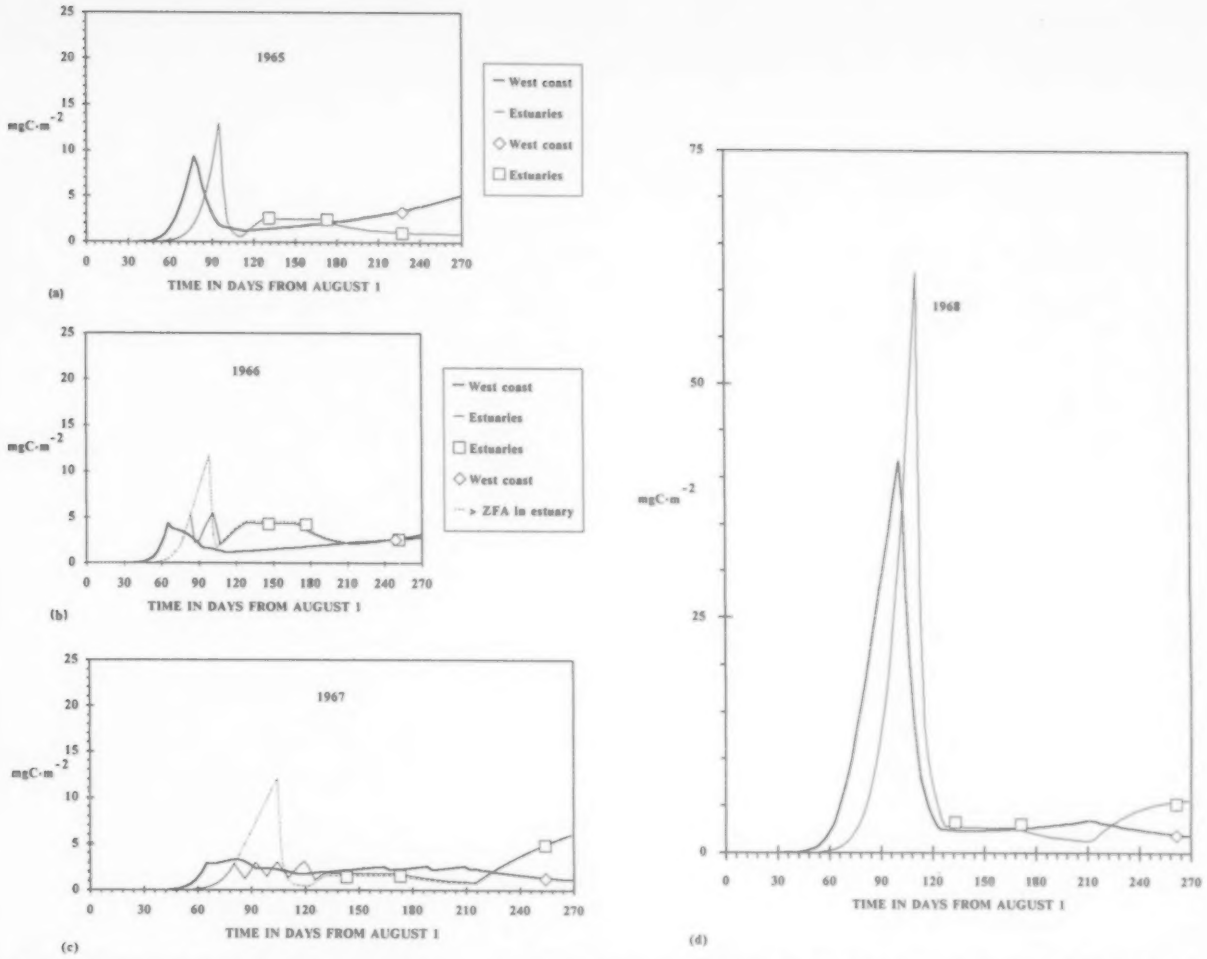


FIG. 10. Model predictions (lines) of large larval biomass in the western sectors from 1965 to 1968 compared with observations (symbols). (a) 1965, western sectors; (b) 1966, western sectors; (c) 1967, western sectors (use the 1966 legend); (d) 1968, western sectors (use the 1965 legend). The single biomass peak in the estuaries for Fig. 10b and 10c is obtained when estuarine ZFA is increased over its value in the coastal waters.

allowed a good test of the model to be made by simulating the years 1966, 1967, and 1968. The 1965 simulation results were not used as a test because information from this year was used to calculate winter starvation parameters k_D and K_{1145T} which were incorporated into the base model. A good fit for the years 1965–68 was obtained by adjusting the relationship between ZFB in the western estuarine and coastal waters (Fig. 10). ZFB in the coastal waters was kept as close to the measured values as possible and ZFB in the estuarine waters was adjusted (see Table 3). ZFA in the western estuaries was also unknown, but it was set equal to the values estimated for the coastal waters in the initial simulations. A higher value for ZFA in the estuaries during 1966 and 1967 caused large larval biomass to reach a single large peak instead of multiple small peaks (Fig. 10b and 10c). The model can accommodate a wide range in the zooplankton population parameters (compare Fig. 10b and 10c with 10d).

Sensitivity Analysis

The sensitivity of the model to variations in the forcing functions, temperature, longshore transport, spawning, and the zooplankton parameters ZFA and ZFB was tested for the base year 1971. The transport sensitivity analysis was performed using two different levels of winter mortality in the eastern coastal area where Sherman's zooplankton measurements showed high winter variability (Fig. 7b and 8b). Sensitivity analysis results are shown as scaled changes in large larval numbers at day 249 of the simulation as a function of the scaled value of one of the forcing functions. Scaled variables were divided by the value they had at the same time in the base run of the model. Thus, small larvae in a simulation run where the scaled value of transport was equal to 1.5 were subjected to a longshore water movement that was 50% greater than the longshore transport in 1971, the base year.

Simulation results indicated that there was an optimum transport for maximum larval survival in the western coastal area which occurred at twice the 1971 flow (Table 4). Longshore transport carried increasing numbers of small larvae from the eastern to the western areas, but it also moved small larvae out of the western area toward Massachusetts Bay. The net effect

TABLE 3. Comparison between estimates of the zooplankton population parameters, ZFA and ZFB, calculated from Sherman (1968, 1970) and values used to simulate larval herring survival from 1965 to 1968.

	ZFA			ZFB		
Year	Eastern coast	Western coast	Western estuary	Eastern coast	Western coast	Western estuary
			Estimates from the data			
1965	0.34	0.50	—	0.90	1.00	—
1966	0.34	0.25	—	0.50	0.40	—
1967	0.18	0.15	—	0.95	1.20	—
1968	3.10	3.10	—	1.60	1.40	—
			Values used in the model			
1965	0.34	0.50	0.50	0.90	1.00	1.30
1966	0.34	0.25	0.25	0.50	0.47	6.00
1967	0.18	0.15	0.15	0.95	1.15	1.55
1968	3.10	3.10	3.10	1.60	1.20	8.00

of increased transport was to monotonically decrease the numbers of small larvae in the western coastal waters, thereby delaying the time when food-limited, density-dependent mortality began. Optimum survival resulted when the minimum large larval biomass occurred closest to the time when the fall movement of large larvae into inshore waters ceased (Fig. 11). Less inshore movement of large larvae occurred after the fall die-off for the optimum than for earlier biomass minima. The next later peak and die-off produced by three times the 1971 transport resulted in less loss of larvae due to migration and a larger minimum biomass, but this minimum was delayed 12 d over the time of minimum biomass at optimum survival. Growth of the herring larvae during the intervening 12 d in the optimum case more than made up for the additional biomass lost from the coastal waters through migration into the estuaries.

In general, the effect of longshore transport on larval survival in the west was not large. Larval survival was increased a maximum of 19% over the base run by the optimum effect described in the previous paragraph. A 90% decrease in transport resulted in only a 5% decrease in larval survival. Larval survival was less sensitive to decreased transport because more small larvae remain in the western coastal waters under these conditions, offsetting the survival advantage of a later die-off. Sensitivity analysis of longshore transport using the low 1972 spawning level (Fig. 9c) showed that larval survival changed by only 2% for both a 90% decrease and a 100% increase in transport. Longshore transport had its major effect on larval survival in the west through dispersing larvae and decreasing density-dependent mortality in years of abundance and in supplying additional larvae from the east to the west in years of scarcity.

The pattern of larval survival in the east changed for different conditions of winter mortality. There was an optimum transport for maximum larval survival at low winter mortality, but when winter mortality was large, survival declined as transport was increased. Decreased transport resulted in minor changes in survival for both levels of east coast winter mortality. Decreased survival of larval herring in the east at increased transports for high winter mortality largely canceled out increased survival at higher transports observed in the western areas (Table 4). This combination of positive and negative effects produced a broad region of transport ranging from plus 100% to minus 90% over which a relatively constant recruitment was maintained. However, for low winter mortality in eastern coastal waters, herring larval survival had an optimum 15% greater than the base run at a 100% increase in longshore transport.

We tested the influence of both the magnitude (Table 5a) and distribution (Tables 5b and 5c) of herring spawning on larval herring survival for high and low levels of winter mortality in the eastern coastal region. A 75% reduction in spawning along the entire coast resulted in a 25% decrease in survival for high winter mortality but only a 16% decrease in survival for low winter mortality. Reduced spawning had a greater effect on larval survival in the eastern areas than in the western areas (Table 5a). Maximum survival in the western area occurred at a 25% reduction in the amount of spawning for both high and low levels of winter mortality in the east. Maximum larval survival in the east occurred at the 1971 spawning level for high winter mortality, but this point shifted to 75% of 1971 spawning for low winter mortality. Larval herring survival was most sensitive to decreased spawning in years of high winter mortality.

TABLE 4. Sensitivity analysis of longshore transport which shows its effect on survival for high and low winter mortality in eastern coastal waters.

		Scaled larval numbers					
	Scaled transport	Eastern coast	Eastern bays	Western coast	Western estuaries	Total No. $\times 10^8$	Scaled number
High winter mortality	0.1	0.95	0.95	0.95	0.96	11.53	0.96
	0.5	0.98	0.98	0.89	0.91	11.26	0.93
	1	1.00	1.00	1.00	1.00	12.07	1.00
	1.5	0.57	0.73	1.08	1.07	11.21	0.93
	2	0.50	0.66	1.19	1.15	11.58	0.96
	3	0.42	0.56	1.08	1.07	10.54	0.87
	4	0.37	0.49	1.00	0.97	9.50	0.79
Low winter mortality	0.1	0.95	0.95	0.95	0.96	11.53	0.96
	0.5	0.98	0.98	0.89	0.91	11.26	0.93
	1	1.00	1.00	1.00	1.00	12.07	1.00
	1.5	1.40	0.86	1.08	1.07	13.01	1.08
	2	1.25	1.06	1.19	1.15	13.87	1.15
	3	1.05	1.04	1.08	1.07	12.82	1.06
	4	0.90	0.90	1.00	0.97	11.45	0.95

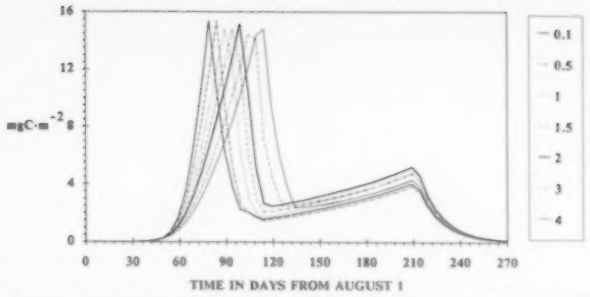


FIG. 11. Model simulations of large larval biomass in the western coastal waters for seven values of longshore transport shown as a fraction of the transport in 1971.

However, when winter zooplankton supplies in the east were good, a 25% decrease in spawning resulted in a 12% increase in overall larval herring survival. A change in spawning quantity resulted in about twice the change in larval survival brought about by an equal percentage change in transport.

In our analysis of the east–west spawning distribution along the Maine coast, the total quantity of spawning was held constant at the 1971 value. The spawning distribution was varied by systematically decreasing the amount of spawning which occurred in one half of the Maine coast and then adding this amount to the other half (Tables 5b and 5c). The model was very sensitive to a decrease in eastern spawning for high winter mortality (Fig. 5b), where a 25% reduction in the amount of spawning resulted in a 50% increase in larval survival in eastern coastal waters and a 33% decrease in the eastern embayments. Additional spawning in the western coastal area resulted in a smaller (8%) decrease in larval herring survival there. The number of surviving larvae in the eastern sectors in good feeding conditions was greatest for a 25% reduction in eastern spawning. However, overall survival decreased monotonically as the fraction of fish spawning in the east declined, but the rate of decrease differed for high and low winter mortality.

Decreasing western spawning while allowing fish that fail to spawn there to spawn in the east resulted in an optimum spawning for maximum survival in the west for both high and low eastern winter mortality (Table 5c). However, in the east, survival decreased monotonically for high mortality, but increased for low winter mortality when more than 50% of the western spawning fish moved to the east. For good winter feeding conditions in eastern waters, additional spawning resulted in a 20% increase in larval survival when 95% of the western spawning fish moved to the east. This increased survival in the east more than compensated for the slight decline in larval survival in the western area due to decreased spawning there. Decreased spawning in the western coastal sector resulted in maximum larval survival at about 50% of the 1971 spawning level. Thus, maximum survival in western waters occurred at an egg production that is 25% less than the amount of egg production that gave maximum survival when the total quantity of spawning was decreased along the entire coast (compare with Table 5a). This change indicates the importance of longshore transport from the east in supplying larvae to the west at low western spawning levels.

The effect of food availability on larval herring survival was tested by varying ZFA and ZFB in a set of model runs. The conditions of high and low winter mortality in the east must be interpreted as changes in predation for this analysis. In the eastern coastal sector, changes in larval survival as a function of autumn food availability proceeded in a series of plateaus with optimum survival between 50 and 100% of the 1971 level. When winter predation mortality in the east was low, higher values of ZFA in the fall resulted in a large increase in larval herring survival. This increase was predicated on the condition that fall feeding was good enough to preclude a strong density-dependent die-off in the eastern coastal waters. A 50% increase in ZFA in the western area produced a maximum larval survival there whereas a 50% decrease in autumn food availability resulted in a 16% decline in larval survival in the western coastal waters and a 10% decrease in the western estuaries. Overall larval survival had an optimum value of ZFA for maximum survival for both high and low winter mortality. However, the optimum ZFA shifted from its 1971 value at high eastern winter mortality to 50% greater than the 1971 value when winter mortality was low (Table 6).

Zooplankton factor B (ZFB) was the most important external factor determining larval herring survival in Maine coastal waters (Table 7). ZFB controlled the winter food resources that determine larval herring carrying capacity after the late fall die-off. A 50% increase in the winter food availability resulted in a 16% increase in overall larval survival for both high and low levels of predation in the eastern coastal waters whereas a 50% decrease in ZFB caused a 39% decrease in larval survival when winter predation was high and a 37% decline when predation was less severe. The lower values of ZFB resulted in a smaller decline in larval survival under conditions of low winter predation mortality than when predation mortality was more severe.

Temperature sensitivity was tested by raising and lowering the 1971 temperature curve by 0.5, 1, and 2°C (Table 8). Winter water temperature was not allowed to fall below −1.5°C in the above calculation. In the eastern embayments, larval survival was better at low winter water temperatures and decreased uniformly as water temperature increased. Temperature was not included as a factor controlling the survival of larval herring in the coastal waters; therefore, survival in these areas did not change as a function of temperature. In the eastern embayments a 1° rise in temperature resulted in a 5% decrease in survival whereas a decrease of 1° caused a 6% increase in survival. We expect that larval survival in the coastal waters would exhibit a temperature sensitivity similar to the eastern embayments, if temperature had been included as a factor controlling large larval biomass there. Temperature affected survival in the western estuaries in a similar manner, but the pattern of decline was masked by low winter survival in the estuaries and a large inshore migration of herring larvae in the springtime.

Winter mortality was used by Graham (1982) as part of an index to predict larval herring survival in Maine coastal waters. The coefficients governing winter mortality in the model were the primary factors which we adjusted initially to obtain a good fit between the base year and the observed data. Table 9 shows that the number of herring larvae that survive is highly sensitive to the value chosen for this coefficient. A 10% decrease in this coefficient gives a 255% increase in survival, and a 10% increase causes a 60% decline in survival. The winter mortality coefficients calculated from 1971 data were used in the simulation of all other years. Because the winter mortality coefficients were constants, the interaction of the forcing functions such as temperature, food supply, etc., determined the severity of winter mortality in years other than 1971.

TABLE 5. Sensitivity analysis of spawning which shows its effect on survival for high and low winter mortality in eastern coastal waters.

		Scaled larval numbers					
	Scaled transport	Eastern coast	Eastern bays	Western coast	Western estuaries	Total No. $\times 10^6$	Scaled number
			(a) Spawning quantity				
High winter mortality	0.1	0.08	0.20	0.89	1.63	12.73	1.05
	0.25	0.18	0.48	0.97	0.95	9.01	0.75
	0.5	0.35	0.49	1.05	1.03	9.95	0.82
	0.75	0.50	0.67	1.14	1.11	11.26	0.93
	1	1.00	1.00	1.00	1.00	12.07	1.00
	1.5	0.95	0.96	0.89	0.91	11.15	0.92
	2	0.92	0.92	0.84	0.89	10.78	0.89
Low winter mortality	0.1	0.20	0.29	0.89	1.63	13.15	1.09
	0.25	0.48	0.70	0.97	0.95	10.08	0.84
	0.5	0.90	0.91	1.05	1.03	11.93	0.99
	0.75	1.27	1.01	1.14	1.11	13.46	1.12
	1	1.00	1.00	1.00	1.00	12.07	1.00
	1.5	0.95	0.96	0.89	0.91	11.15	0.92
	2	0.92	0.92	0.84	0.89	10.78	0.89
			(b) Eastern spawning				
High winter mortality	0.01	0.00	0.02	0.81	0.85	6.67	0.55
	0.05	0.03	0.10	0.84	0.90	7.32	0.61
	0.1	0.08	0.20	0.84	0.89	7.56	0.63
	0.25	0.18	0.48	0.81	0.86	8.22	0.68
	0.5	0.35	0.49	0.84	0.88	8.69	0.72
	0.75	0.50	0.67	0.92	0.92	9.76	0.81
	1	1.00	1.00	1.00	1.00	12.07	1.00
Low winter moratlity	0.01	0.02	0.03	0.81	0.85	6.73	0.56
	0.05	0.10	0.15	0.84	0.90	7.56	0.63
	0.1	0.20	0.29	0.84	0.89	8.00	0.66
	0.25	0.48	0.70	0.81	0.86	9.28	0.77
	0.5	0.90	0.91	0.84	0.88	10.67	0.88
	0.75	1.27	1.01	0.92	0.92	11.96	0.99
	1	1.00	1.00	1.00	1.00	12.07	1.00
			(c) Western spawning				
High winter mortality	0.01	0.87	0.88	0.97	0.97	11.29	0.94
	0.05	0.88	0.89	0.97	0.97	11.33	0.94
	0.1	0.88	0.89	1.00	0.97	11.36	0.94
	0.25	0.88	0.90	1.00	0.98	11.49	0.95
	0.5	0.90	0.92	1.14	1.12	12.64	1.05
	0.75	0.93	0.94	1.05	1.04	12.14	1.01
	1	1.00	1.00	1.00	1.00	12.07	1.00
Low winter mortality	0.01	1.22	1.15	0.97	0.97	12.56	1.04
	0.05	1.20	1.20	0.97	0.97	12.63	1.05
	0.1	1.17	1.16	1.00	0.97	12.53	1.04
	0.25	1.07	1.07	1.00	0.98	12.24	1.01
	0.5	0.90	0.92	1.14	1.12	12.64	1.05
	0.75	0.93	0.94	1.05	1.04	12.14	1.01
	1	1.00	1.00	1.00	1.00	12.07	1.00

Discussion

The energy circuit model served as a heuristic tool that allowed us to increase our understanding of larval herring survival in Maine coastal waters. The evaluated model provided a framework for organizing processes and analyzing information about the relative importance of the various environmental and biological factors which determine larval herring recruitment. This concrete formulation for larval recruitment forced us to think in holistic and quantitative terms about how the herring recruitment process works and whether or not any particular formulation of ideas was plausible. Computer simulation of the model demonstrated that we did not yet understand or include in our model some of the fundamental processes con-

TABLE 6. Sensitivity analysis of ZFA which shows its effect on survival for high and low winter mortality in eastern coastal waters.

		Scaled larval numbers					
	Scaled ZFA	Eastern coast	Eastern bays	Western coast	Western estuary	Total No. $\times 10^8$	Scaled number
High	0.1	0.80	0.34	0.59	0.59	6.89	0.57
winter	0.25	0.80	0.35	0.89	0.89	9.25	0.77
mortality	0.5	1.00	0.46	0.84	0.90	9.92	0.82
	0.75	1.00	0.65	0.86	0.89	10.35	0.86
	1	1.00	1.00	1.00	1.00	12.07	1.00
	1.5	0.63	0.83	1.05	1.04	11.33	0.94
	2	0.63	0.84	0.86	0.85	9.85	0.82
Low	0.1	.93	0.39	0.59	0.59	7.24	0.60
winter	0.25	0.93	0.40	0.89	0.89	9.61	0.80
mortality	0.5	1.00	0.46	0.84	0.90	9.92	0.82
	0.75	1.00	0.65	0.86	0.89	10.35	0.86
	1	1.00	1.00	1.00	1.00	12.07	1.00
	1.5	1.58	1.56	1.05	1.04	14.79	1.23
	2	1.58	1.56	0.86	0.85	13.31	1.10

TABLE 7. Sensitivity analysis of ZFB which shows its effect on survival for high and low winter mortality in eastern coastal waters.

		Scaled larval numbers					
	Scaled ZFB	Eastern coast	Eastern bays	Western coast	Western estuaries	Total No. $\times 10^8$	Scaled number
High	0.1	0.12	0.12	0.16	0.15	1.73	0.14
winter	0.25	0.22	0.22	0.46	0.43	4.29	0.36
mortality	0.5	0.47	0.47	0.70	0.68	7.36	0.61
	0.75	0.73	0.74	0.86	0.85	9.78	0.81
	1	1.00	1.00	1.00	1.00	12.07	1.00
	1.5	1.50	0.80	1.19	1.19	14.00	1.16
	2	1.98	0.92	1.32	1.37	16.46	1.36
Low	0.1	0.53	0.50	0.16	0.15	3.38	0.28
winter	0.25	0.53	0.51	0.46	0.43	5.56	0.46
mortality	0.5	0.53	0.52	0.70	0.68	7.60	0.63
	0.75	0.73	0.74	0.86	0.85	9.78	0.81
	1	1.00	1.00	1.00	1.00	12.07	1.00
	1.5	1.50	0.80	1.19	1.19	14.00	1.16
	2	1.98	0.92	1.32	1.37	16.46	1.36

TABLE 8. Sensitivity analysis of temperature effects on larval herring survival caused by deviations from the monthly 1971 average.

	Scaled larval numbers					
Temp. change	Eastern coast	Eastern bays	Western coast	Western estuaries	Total No. $\times 10^8$	Scaled number
−2	1.00	1.12	1.00	1.03	12.61	1.04
−1	1.00	1.06	1.00	1.01	12.27	1.02
−0.5	1.00	1.03	1.00	0.99	12.06	1.00
0	1.00	1.00	1.00	1.00	12.07	1.00
0.5	1.00	0.97	1.00	1.01	12.08	1.00
1	1.00	0.95	1.00	1.02	12.11	1.00
2	1.00	0.90	1.00	1.00	11.84	0.98

TABLE 9. Sensitivity of larval herring survival to changes in the winter mortality coefficient.

Scaled mortality	Scaled larval numbers: Eastern coast	Eastern bays	Western coast	Western estuaries	Total No. $\times 10^8$	Scaled number
0.9	1.65	0.85	4.35	3.14	30.79	2.55
1	1.00	1.00	1.00	1.00	12.07	1.00
1.1	0.60	0.67	0.19	0.28	4.38	0.40
1.25	0.28	0.36	0.00	0.07	1.87	0.16
1.5	0.08	0.14	0.00	0.03	0.71	0.06
2	0.00	0.03	0.00	0.01	0.15	0.01

trolling larval herring recruitment in Maine waters. Through a process of interaction between the field scientist and the modeler, we made a series of alterations to the original model that resulted in the inclusion of additional factors and processes as shown in Fig. 2 and 4. As additional relevant processes were included in our model formulation, the fit of the model output to data from the base year progressively improved (see Fig. 7 and 8). This interaction resulted in the development of three working hypotheses which are possible to test in further field research.

Working Hypotheses

The critical period hypothesis was first developed by Hjort (1914) and has been a dominant idea in fisheries science since that time. Although this concept most often associates a critical period of increased larval mortality with the time of yolk sac resorption and first feeding, there is no reason why there could not be several critical times in the larval life of a fish when food availability sharply limits survival. The modeling process led to the recognition that the relationship between larval herring in Maine waters during late fall and early winter and their zooplankton food may be a critical factor determining the survival of herring larvae in the following spring. We hypothesized that early winter was a critical period during which the larval herring were forced to switch from copepodites and nauplii, their dominant autumn food, to small copepods such as *Pseudocalanus* and *Oithona* which were the major constituents of their winter food. This switch was forced by decreasing supplies of small food items and facilitated by the increasing average size of the herring larvae which were just large enough to consume small copepods by early winter. This hypothesis can easily be tested with data gathered during the proposed critical time.

Iles and Sinclair (1982) hypothesized that genetically distinct herring stocks were associated with geographically distinct larval retention areas. In addition, they suggested that herring stock size depended on the larval retention area available to support the larval and postlarval stages. Observations in eastern Maine coastal waters confirmed the association of spawning herring stocks with hydrographically distinct areas of little horizontal flow (Watson 1936; Sinclair and Iles 1985; Chenoweth et al. 1989). Herring larvae spawned in eastern Maine appeared to use the entire coastal area shoreward of the 100 m isobath as their nursery area (Graham 1982; Townsend et al. 1986; Chenoweth et al. 1989). Chenoweth et al. (1989) observed that larvae hatched in the Grand Manan and eastern Maine spawning area at the same time had different horizontal displacements from the spawning area. Some larvae remained in the area up to a month after spawning whereas others were transported up to 100 km down the coast. Because herring spawning occurs

TABLE 10. Areas of the model sectors and their relative contributions to recruitment based on area and volume.

Spatial sector	Area (m^2)	Volume (m^3)	Scaled area	Scaled volume
Eastern coast	3.05×10^9	1.83×10^{11}	1.0	1.0
Western coast	2.63×10^9	1.58×10^{11}	0.86	0.86
Eastern embayments	1.93×10^9	0.77×10^{11}	0.63	0.42
Western estuaries	1.48×10^9	0.59×10^{11}	0.49	0.32

in regions of so-called dead water where little horizontal flow exists, we found it necessary to hypothesize a release mechanism to explain these observations. We hypothesized that the release of larvae from eastern Maine spawning grounds is mediated by wind events which generate horizontal flows that carry herring larvae out of the regions of usually dead water and into the counterclockwise residual flow that moves from northeast to southwest along the Maine coast. After a wind event subsides, tidal mixing acts to restore the region of homogenous water over the spawning grounds. This hypothesis can account for the different transport histories of the same larval cohort as well as the variation in the transport times of different larval cohorts seen by Graham and Townsend (1985). This working hypothesis could be tested in the field, but the monetary cost would be great. A more logical method for testing this idea is to develop a mathematical model of the coastal circulation in eastern Maine.

Finally, model simulations indicated that larval behavior might be an important factor in determining the distribution of herring larvae along the Maine coast. Inshore movements of large larvae in both fall and spring were necessary for the model to reproduce the observed spatial distribution of larval biomass. The fall movement of large larvae into inshore waters has been documented at the Sheepscot Estuary and in Sullivan's Harbor embayment (Townsend and Graham 1981; Graham et al. 1984). Townsend et al. (1989) examined the strontium/calcium ratio in the otoliths of larval herring found in estuarine waters in spring and determined that these fish had recently been exposed to water of higher temperature. Because warmer water temperatures were found offshore at this time of year, they concluded that this evidence supports an inshore spring movement of herring larvae from the coastal to estuarine waters.

Predicting Larval Herring Survival

Larval herring were measured in the field as a concentration in numbers of larvae per volume filtered. Each of our model sectors had a different area and volume (Table 10). Movement of larvae from coastal to estuarine waters must take into account the fact that the same number of larvae are contained in a smaller

area and volume after inshore migration, thus leading to greater concentration. Each area was multiplied by the estimate of spring abundance in biomass per square metre and divided by the average larval size in inshore or offshore waters to estimate the contribution to recruitment from each sector. When these assumptions were applied, the eastern coastal sector accounts for 1.58 times the larval herring produced by the eastern embayments, and the western coastal waters produce 1.76 times more larvae than the western estuaries for the same average biomass measured per square metre of sea surface. This observation is simple but it is a central point in interpreting the observed spatial and temporal distribution of herring larvae along the Maine coast and for accurate prediction of overall larval survival.

Values were calculated for the number of larvae surviving in each sector and the total number of larvae surviving in Maine coastal waters on April 12 in each of the nine years studied (Table 11). Model simulations showed that in all years except 1966, most larvae survived the winter in the coastal waters. However, observations of larval herring concentrations from the spring cruise data (Graham 1982 and Table 11) indicate that the relative distribution of larvae between the estuarine and coastal waters was quite variable from one year to another. Further investigation of the behavioral ecology of herring larvae is necessary to discover the cause of this variable spring movement.

The last two columns in Table 11 compare the normalized number of herring larvae surviving in the model to the normalized predictive index used by Graham (1982). The model index includes a prediction of larval survival in the eastern areas for which Graham (1982) had no information. Graham's index is an equally weighted average of survival in the western coastal and estuarine waters. His index weights observations in the western estuaries too heavily in years when there is a large difference between larval concentrations in the estuarine and coastal waters. This is evidenced by the fact that Graham's 1982 index is different from larval survival predicted by the model in 1971 and 1965 when larval survival in the estuaries was much larger and smaller than the average. The model index is most different from Graham in 1966 when survival in the west was fairly good but survival in the eastern areas was poor. The model estimate of low survival in the east for 1966 was based on zooplankton measurements of Sherman (1968) from which estimates of ZFA and ZFB for that year were calculated. We conclude that the only reliable estimates of overall larval survival are obtained by monitoring the entire coast each spring and weighting the resulting observations by area regardless of the technical difficulties involved in comparing data gathered by different sampling methods (see Graham 1982). The alternative to a spring larval assessment is a spring brit assessment which is also costly and as yet not as reliable as the larval assessment.

The Maine harvest of 2-yr-olds was plotted against the model estimates of the number of larvae that survived to metamorphosis (Fig. 12). The optimum curve formed by the data (omitting 1966 for the moment) implies that density-dependent mortality occurred in the brit stage for high survival in the larval stage. An obvious hypothesis is that recruitment to the herring fishery is largely determined by processes in the larval stage of their life history when survival is below a threshold. However, when survival exceeds this threshold, recruitment is not established until the brit year. This relationship suggests that for herring the critical stage at which recruitment is established shifts from the larval stage to the early juvenile stage in some years. This result fits well with Houde (1987) who deduced that herring recruitment was primarily determined in the larval stage but could be fixed in the brit stage some of the time. Graham and Sherman (1987) have proposed a paradigm of shifting critical stages for herring recruitment which our results generally support. The large Maine herring harvest in 1966 (Fig. 12) has been attributed to a larger than usual influx of fish from the Georges Bank population (Graham and Sherman 1987). Because the model was able to accurately predict spring survival in all years examined, including 1966, we conclude that movement of fish from Georges Bank to the Maine coast must have occurred after metamorphosis.

Density-Dependent Predation on Brit

There is some evidence to support the working hypothesis that density-dependent predation on brit is an important factor in determining herring recruitment in some years. Chenoweth et al. (1989) described the particularly successful spawning of the 1986 herring year-class in Maine coastal waters. In addition, the spring assessment cruise in 1987 recorded the largest quantities of larval herring ever observed along the Maine coast (Stevenson et al. 1989). Thus, we know that 1986 was a particularly successful year-class on the Maine coast up to metamorphosis, and yet the Maine harvest of 2-yr-olds in 1988 was not much larger than the 1987 harvest. During the summer of 1987, one of us sampled a small number of party boats with the help of Earthwatch volunteers to obtain an index of pelagic predation on the 1986 year-class. We found that both mackerel

TABLE 11. Model predictions of the number of larval herring that survive to metamorphosis in each of the four spatial sectors. Normalized total survival predicted by the model is compared with the index of spring survival in Graham (1982).

	Larval numbers × 10^8						
Year	Eastern coast	Eastern bays	Western coast	Western estuaries	Number × 10^8	Scaled total	Scaled index (Graham 1982)
1965	3.17	1.32	11.40	0.84	16.73	0.90	0.69
1966	1.60	0.73	6.79	2.30	11.41	0.61	0.91
1967	3.02	0.50	3.42	4.37	11.31	0.61	0.75
1968	5.21	2.39	5.23	3.84	16.67	0.90	0.87
1969	1.45	0.57	3.19	2.52	7.73	0.42	0.53
1970	3.14	1.29	3.67	5.32	13.42	0.72	0.92
1971	1.60	2.43	0.69	7.12	11.83	0.64	1.00
1972	3.11	1.24	12.68	1.58	18.60	1.00	0.95
1973	2.28	0.87	5.05	2.05	10.25	0.55	0.60

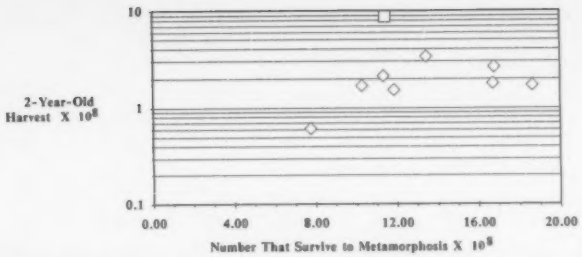


FIG. 12. Comparison of the model predictions for larval herring survival on the Maine coast from 1965 to 1973 with the Maine harvest of 2-yr-old fish 2 yr later. All years except 1966 are plotted as diamonds.

and bluefish were feeding heavily on brit herring along the Maine coast in the summer of 1987. Langton et al. (1989) found that brit herring were present in the diets of four of the six most abundant groundfish in the Sheepscot Estuary. Longhorn sculpin was the most abundant fish in his samples and was typical of those groundfish that were feeding on herring. Four hundred and sixty-eight longhorn sculpin were sampled of which 3.2% contained brit herring comprising 12.8% of their stomach contents by weight (Langton et al. 1989). Fish food data from the Northwest Atlantic in Mauer and Bowman (1975) show that the quantity of fish in the diet of the Sheepscot demersal fish is not unusually large; however, the presence of Atlantic herring as a major component of their diet is most unusual. A working hypothesis that explains our observations on the 1986 herring year-class is that opportunistic pelagic and demersal fish species feed preferentially on a food fish such as herring if it exceeds an abundance threshold.

Sissenwine (1984) proposed that predation on post larval fish was a major factor determining recruitment. Winters (1976) found that mackerel displaced herring as the dominant pelagic species in the southern Gulf of St. Lawrence during the 1960's. He attributed this change to a complex set of factors mediated by competition for the limited pelagic carrying capacity of the southern Gulf. Lett and Kohler (1976), following Ricker (1954), suggested that predation by mackerel and cannibalism by older herring would be the ultimate population control on juvenile herring. Skud (1982) showed that herring and mackerel have alternately dominated pelagic biomass along the eastern coast of North America over the past 200 yr. Because herring lost dominance to mackerel in the late 1960's (Skud 1982), most of the years examined in our model occur during the period when mackerel predation on juvenile herring could be an important factor controlling recruitment.

Factors Controlling Larval Herring Survival

External forcing functions controling larval herring survival were grouped into primary and secondary factors according to the sensitivity analysis results. Primary factors caused a 20% or greater change in larval survival over the range from a 50% increase to a 50% decrease in the factor. Secondary factors brought about less than a 10% change in survival over a similar range of the causative factor. Houde (1987) demonstrated that small changes in growth rate, mortality, and stage duration in the early life history of fish can bring about large variations in recruitment. Our sensitivity analysis examines the role of external factors in causing variations in larval survival that may be mediated by biological factors such as growth rate and mortality.

TABLE 12. Changes in the total larval herring survival caused by equal percentage changes in the external forcing functions.

	High winter mortality		Low winter mortality	
Forcing function	+50%	−50%	+50%	−50%
Longshore transport	−7%	−7%	+8%	−7%
Total spawning	−8%	−7%	−8%	+12%
Eastern spawning	+5%	−28%	+5%	−12%
Western spawning	−28%	+5%	−12%	+5%
Zooplankton factor A	−6%	−18%	+23%	−18%
Zooplankton factor B	+16%	−39%	+16%	−37%

Variation in the primary factors, ZFA and ZFB, which represent autumn and winter food supplies caused a greater change in survival than did similar variation in spawning quantity and distribution (Table 12). Variations in the autumn food supply produced only about half the effect of varying winter food supply. In the western coastal sectors, an optimum pattern of larval survival emerged because larval biomass in the western coastal zone was in part determined by the balance between biomass lost due to inshore larval migration and biomass gained through growth in the more favorable winter mortality regime following the fall die-off. An earlier die-off resulted in more losses through inshore migration, but also a greater time for growth when mortality was low. Similarly, Houde (1987) found that small changes in average stage duration could produce large variations in survival for the five species of fish larvae he examined.

Increased autumn food supplies led to a considerable increase in larval survival in the eastern coastal waters for low winter mortality (Table 6). Increased survival was observed in this case because autumn food supplies were large compared with larval biomass so that the usual fall die-off did not occur in the eastern coastal waters, and greater numbers of larvae survive to winter when food supplies were assumed to be good. The ratio of zooplankton abundance to larval herring abundance in the eastern coastal waters was low enough to cause a fall die-off from 1962 to 1967 (Graham et al. 1972a). It may be possible for large zooplankton populations in the east to produce exceptional larval herring survival, but we have no empirical evidence to support this model result.

Sensitivity analysis demonstrated that a strong relationship between larval herring survival and their zooplankton food existed. This result highlights the need for further investigation of the factors that control zooplankton populations and productivity in well-mixed as well as stratified waters. Research on phytoplankton and zooplankton in the Bay of Fundy as described by Daborn (1986) provides a good model to guide research in Maine coastal waters. He hypothesized that in well-mixed waters, phytoplankton and zooplankton crops are poor in part due to grazing by the benthos. In contrast, stratification releases plankton populations from predation which increases plankton production and decreases production of the benthos. This conceptual model is a plausible working hypothesis to guide field research on the factors that produce abundance or scarcity in eastern Maine zooplankton populations.

Over the range of values tested in the model, the east-west distribution of spawning was more important than the quantity of spawning in determining larval survival. Survival was particularly sensitive to a decrease in spawning in the eastern coastal waters, but was much less sensitive when spawning in

the west was decreased (Table 12). This relationship occurs because herring larvae do not enter Maine waters from Canadian waters to the east (Chenoweth et al. 1989); therefore, local spawning is the only supply of larvae to the eastern coastal waters and embayments. Larvae are supplied to western waters in the southwestward residual flow, so loss of local spawning in the west is not as critical in maintaining the supply of larvae there. Also, feeding conditions in the western region are often good enough to result in good recruitment even when spawning is small.

The number of larvae that survive along the Maine coast in a given year is linked to successful and well-distributed herring spawning. The current State of Maine management policy for herring relies on a series of spawning closures which take effect at different times along the coast. Our study indicates that this policy should promote the production of good herring year-classes in most years (those with high winter mortality in the east). While spawning is not the most important factor determining year-class strength, it plays a significant role, and the fishing activity which can disrupt it is subject to regulation.

Temperature and longshore transport were shown to be secondary factors by our sensitivity analysis. These secondary factors might have their most important impact on larval survival through interaction with primary factors. For example, temperature and predation interact to cause winter larval mortality in the western estuaries. Predation on herring larvae is not explicitly evaluated in the sensitivity analysis, but our simulations implied that variations in predation can generate large changes in larval survival. Thus, the greatest temperature effects may not be manifested except with changes in the predation regime (Graham et al. 1990). A secondary factor may also have a primary effect if it operates in a manner different from its expression in the model, e.g. lethal temperature limits are not part of our temperature formulation. Longshore transport also may have its largest effect through interaction with spawning distribution, since the dispersion of larvae along the coast can change survival. The potential effect of catastrophic transport events such as the one observed by Brooks and Townsend (1989) in carrying herring larvae away from the Maine coast was not considered in our analysis. Longshore transport could be either more or less important than estimated, if small herring larvae can control their vertical distribution as observed by Stephenson and Power (1988).

Model Validation

Coefficients, such as winter mortality, are calculated from the values assigned to storages and flows in the model evaluation. These coefficients are constants only if all factors that cause major variations in the flows they govern are included in the model. The model was able to accurately predict larval herring survival in a semiindependent test (Sherman's 4-yr mean biomass was used to evaluate 1971) using Sherman's zooplankton data from 1966, 1967, and 1968. The small changes necessary to produce this result indicated that the winter mortality was adequately evaluated in the model (Table 3). The data from 1969, 1970, and 1973 were used to demonstrate the ability of ZFA and ZFB to control larval herring survival in years when the environmental forcing functions differed. Data from 1972 provided an independent test of the model for a year over which we had information similar to the base year, 1971. Simulation of the model using 1972 conditions resulted in an excellent agreement between model and data for the western sectors as shown in Fig. 9b. Only the autumn mortality coefficient had to be changed to give a good fit to the 1972 data from the eastern sectors as seen in Fig. 9a. Eastern spawning in 1972 was much smaller than in 1971, and it is plausible that the factors controlling larval herring mortality may differ in kind or intensity for large and small larval populations. This combination of tests demonstrated that the model gave a reasonable description of the herring recruitment process along the Maine coast for the years 1965–73.

Prediction of year-class strength which is under the influence of so many variables is a difficult task which is not easily accomplished. An additional degree of difficulty in prediction is added by the fact that our analysis of the relationship between herring surviving to metamorphosis and the Maine harvest of 2-yr-olds demonstrated that herring year-class stength is probably established in the brit year part of the time. Also, in some years, brit herring from the next larger system may dominate the Maine coastal population and mask the actual Maine coast production of a given year-class (e.g. the apparent influx of juveniles from Georges Bank in 1966). A further complicating factor may lie in the ability of the herring themselves to change their behavior (e.g. late spawning, Graham 1982) and thereby alter the nature of the recruitment process. In the future we plan to test our model with additional years in which Maine herring have shown a lengthened spawning season.

Conclusions

From the modeling process we conclude the following, although we recognize that some of these statements are really working hypotheses which can not be verified without further field study.

(1) Larval herring recruitment in Maine coastal waters is the result of a complex interaction of many processes, no one of which is truly dominant.

(2) Two-year-old recruitment to the Maine herring fishery is established in the larval stage in some years and not until the brit stage in other years.

(3) Larval food supply in autumn and winter, along with the quantity and distribution of spawning, are primary factors controlling herring recruitment for those years when the larval stage is critical.

(4) When larval survival is above a threshold, density-dependent predation on brit can reduce the year-class size. We assume that the brit become the food of choice for opportunistic pelagic and demersal predators when brit exceed an abundance threshold.

(5) Temperature and longshore transport are secondary factors determining larval survival that may be most important through their interaction with primary factors.

(6) An estimate of the absolute number of larvae that survive along the Maine coast should be used to predict subsequent recruitment. The coastal and inshore areas do not make equal contributions to recruitment, and survival in one area is not always a good index of survival in other areas.

(7) In most years, more larvae survive the winter in the coastal areas than in the estuaries and embayments.

(8) The distribution of larvae along the Maine coast in springtime is largely a function of the variable inshore movement of larvae.

(9) Herring larvae may be released from their eastern spawning areas by horizontal, wind-driven water movements that result in the unique temporally pulsed and spatially patchy dis-

tribution of larvae observed in the autumn along the Maine coast.

(10) Any contribution of the Georges Bank herring stock to recruitment on the Maine coast probably occurred after metamorphosis.

(11) Present Maine herring management regulations which implement a spawning closure on fishing activity at various times and places along the Maine coast in autumn appear to be a reasonable policy that should increase herring recruitment on average.

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Appendix

The forcing functions, storages, and flows of the larval herring recruitment model (Fig. 2) are evaluated below. A description of how each function or value was obtained is given along with the assumptions, formulae, and references necessary to complete the calculation.

Forcing Functions

J_{Ii} is the monthly average solar radiation received on a horizontal surface from August to April. List (1984) gave the formula

$$(1) \quad Q = Q_0(0.61S + 0.35)$$

for the calculation of the average solar radiation, Q, given the fraction of possible direct solar radiation, S, and the solar radiation received on cloudless days, Q_0. The NOAA local climatological data sheets for Portland, ME, were used to obtain an estimate of S in the western coastal section. A monthly comparison of S for the years 1949–52 between Portland and East-

port, ME, was used to adjust the Portland data to reflect the greater average cloudiness of weather in the eastern coastal region. Q_0 was estimated from List (1984) using an average latitude of 44°30′ for the eastern sector and 43°50′ for the western sector along with the assumption that the atmospheric transmission coefficient is 0.9 on cloudless days. The western and eastern solar radiation functions were used for both coastal and estuarine spatial cells in their respective regions.

J_{Ti} is the effective volume transported each day by the tide. The salt balance equation was used to estimate an effective tidal exchange volume (McKellar 1975) which was found to be 62% of the actual volume for the eastern half of the Penobscot Bay using data in Meade (1970). The tidal embayments along the eastern coast of Maine were assumed to have similar exchange properties. The tidal prism of the eastern Maine estuarine area outlined in Fig. 1 is approximately 3.23×10^9 m^3. The volume exchanged per tide is then 2.0×10^9 m^3 times 1.93 tides per day which gives about 3.9×10^9 m$^3 \cdot$d^{-1} transported by the tide in the eastern sector. In the western sector the salt balance equation was used to estimate tidal exchange coefficients of 0.03 for the Sheepscot estuary and 1 for the western Penobscot Bay from information given in Garside et al. (1978), Meade (1970), Haefner (1967), and Apollonio and Applin (1972). If Casco Bay has exchange characteristics similar to the eastern embayments, an area-weighted average along the western coast gives a tidal exchange coefficient of 0.7. By a calculation similar to that used for the eastern coast, this exchange coefficient gives a tidal transport of 2.7×10^9 m$^3 \cdot$d^{-1} for the western section.

J_C is the volume transport of the current along the Maine coast. The approximate cross-sectional areas of the three vertical sections from shore to the 100-m isobath which form the boundaries of the eastern and western sections in Fig. 1 are Gran Manan (1.98×10^6 m^2), Matinicus (1.98×10^6 m^2), and Cape Porpoise (1.14×10^6 m^2). In addition, we estimated the cross-sectional area of the coastal shelf at Monhegan Island to be about 1.5×10^6 m^2 which allowed us to estimate the magnitude of offshore water movement between Mt. Desert Island and Monhegan Island. A current speed of 8 km$\cdot$d^{-1} off Petit Manan in September was estimated from Bumpus and Lauzier (1965) and applied to the waters moving through the Gran Manan and Matinicus coastal cross-sections. The volume transport into and out of the eastern coastal section is about 1.58×10^{10} m$^3 \cdot$d^{-1} based on the above assumptions. Vermersch et al. (1979) measured an average residual current speed of 6.65 km$\cdot$d^{-1} at 33 m near Monhegan Island from November to January 1975. If this estimate is typical of fall velocities there, the volume transport into the western sector is 1.0×10^{10} m$^3 \cdot$d^{-1}. We assume that the difference between these two transports, or 0.58×10^{10} m$^3 \cdot$d^{-1}, moves offshore at the boundary between sections. The residual fall current velocity through the Cape Porpoise section was estimated to be 8 km$\cdot$d^{-1} from their October measurements off Casco Bay. Transport out of the western sector was 0.91×10^{10} m$^3 \cdot$d^{-1} which is approximately equal to the transport through the Monhegan cross-section.

J_W is the volume transported inshore by the wind. The average monthly wind velocities were obtained from the local climatological data for Portland. Wind stress was calculated as in Saunders (1977). Saunder's data showed that the average wind stress along the eastern and western Maine coasts in autumn is about equal. Therefore, Portland wind data were used for both the eastern and western sectors in the model. An Ekman transport velocity at the surface was calculated using the formulas in Sverdrup et al. (1942, p. 493 and 496) assuming an average water depth of 60 m for the Maine coast and latitude 44°. For an 11-yr (1965–73, 1976, and 1981) average autumn wind speed of 3.6 m$\cdot$s^{-1}, the depth of frictional resistance to the wind is about 30 m using formula (XIII, 81) in Sverdrup et al. (1942). The linear dimension of the two coastal sections is about 145 000 m giving a cross-sectional area for transport of 4.35×10^6 m^2. The calculated values for Ekman transport velocity at the surface were depth averaged to 30 m and multiplied by the calculated cross-sectional area to obtain transport volumes. These volumes were decreased by one half because roughly half of the autumn winds along the Maine coast result in onshore-directed movements of water at the surface (see fig. 4 in Graham 1970).

J_{NE} is the additional longshore volume transport generated by the interaction of northeast winds with the southwestward flowing circulation and the Maine coastal topography. All days with resultant wind velocities of 1.8 m$\cdot$s^{-1} or greater and wind direction from 30 to 60° were recorded from the local climatological data for Portland. Ekman transport velocities at the surface were calculated as above. The effects of wind were checked by reference to Greenberg (1983) who used a mathematical model to show that a 0.5 dyne$\cdot$cm^{-2} wind stress could develop a southwestward flowing circulation averaged along the Maine coast of about 12 cm$\cdot$s^{-1}. The calculated surface Ekman transport velocity for a similar wind stress was 5.9 cm$\cdot$s^{-1}. The average residual current is about 4.0 cm$\cdot$s^{-1}, leaving 2.1 cm$\cdot$s^{-1} to be accounted for by the interactive effect of the northeast wind with the Gulf of Maine topography as shown in Greenberg's model. Thus, the calculated northeast transports were increased by 33% to account for the synergistic effect of the current, wind, and coastal bathymetry. These velocities were depth averaged and multiplied by the width of the shelf to the 100-m isobath times the depth of frictional resistance to obtain an estimate of the additional southwestward volume transport by the northeast wind. J_{WE} is the time series of wind events from any direction where average velocity for the day was 5 m$\cdot$s^{-1} or greater.

J_{Temp} is the seawater temperature in the estuarine waters of the Maine coast. Seawater temperature at Boothbay Harbor, ME, was obtained from Welch and Churchill (1984).

J_{Yi} is the influx of yolk sac larvae to the water column from egg beds along the Maine coast. Graham et al. (1973) supplied data which were used to estimate the distribution of herring spawning from Cape Cod to Gran Manan as 25% eastern Maine, 25% western Maine, and 50% further to the southwest. The number of eggs produced in each sector was obtained by taking the VPA estimates of 4+ stock size from Fogarty and Clark (1983) and multiplying by the age-specific fecundity data of Morse and Morris (1981) and then dividing by 4 to obtain the egg production in each sector of the Maine coast. Mortality in the egg stage is estimated at about 10% (Caddy and Iles 1973) and mortality of the yolk sac stage is assumed to be another 10%. Numbers of yolk sac larvae were converted to milligrams of carbon per square metre assuming a 7 mm average size for the larvae and using Chenoweth (1970) to estimate dry weight and then multiplying by 0.55 carbon to dry weight ratio and dividing by the area of the coastal section.

ZFA and ZFB are indices that represent the relative suitability of the zooplankton population as food for larval herring. Copepod naupli and small copepodite numbers were used as an estimator for ZFA. Since small zooplankton were not adequately sampled by Sherman (1968, 1970), the total number of

adult copepods present in Maine coastal waters during the autumn was used to estimate the relative change in the numbers of copepod juvenile stages. The value of ZFA and ZFB in any year is scaled by the appropriate average value for the years 1965–68. We assumed that ZFA in 1971 equals the 1965–68 average value. ZFB is the quantity of small copepods present in Maine coastal waters during early winter that are small enough to be consumed by larval herring. ZFB is calculated as the average of the scaled value of the total number of copepods in the size range 0.6–2.0 mm and the scaled value of the numbers of *Pseudocalanus minutus* and *Oithona simulus* which are the principal food of Maine larval herring in early winter according to Sherman and Honey (1971).

Storages

P_i is the amount of phytoplankton in the water in any model sector. Measurements of chlorophyl *a* in the coastal water were taken from Apollonio and Applin (1972). An average carbon to chlorophyl ratio of 45:1 was used (Parsons and Takahashi 1973). Chlorophyl is assumed to be present in significant quantities to an averge depth of 25 m in the estuarine waters and to a depth of 50 m in coastal waters. Averaging Apollonio and Applin's September 1971 transects through the eastern and western areas gives $P_3 = 6.2$ g C·m^{-2}, $P_4 = 6.4$ g C·m^{-2}, $P_2 = 3.7$ g C·m^{-2}, and $P_1 = 4.5$ g C·m^{-2}.

Z_i is the amount of zooplankton in the water for each model sector. No zooplankton data were available for 1971; therefore, we chose to use the average zooplankton biomass from Sherman (1966, 1968, 1970). Since Sherman used 0.366-mm-mesh nets which did not sample the microzooplankton, we adjusted his volumes according to the observations of Beers and Stewart (1969). Their maximum microzooplankton estimate, 43% of the macrozooplankton volume, was added to the zooplankton biomass in the eastern sectors because the data of Bigelow et al. (1940) indicated that planktonic protozoans are most abundant in the eastern sector in fall. We added 25% of the macrozooplankton volume to account for the microzooplankton in the western sectors. The zooplankton biomass in each sector was $Z_4 = 100$ mg C·m^{-2} of which 20 mg is microzooplankton, $Z_3 = 243$ mg C·m^{-2} of which 49 mg is microzooplankton, $Z_2 = 27$ mg C·m^{-2} of which 9.3 mg is microzooplankton, and $Z_1 = 80$ mg C·m^{-2} of which 16 mg is microzooplankton. Conversions from displacement volume to milligrams of carbon were made using the formula of Wiebe et al. (1975).

S_i and L_i are, respectively, the amounts of small (7–15 mm) and large (>15 mm) herring larvae present in each sector. Data from Graham et al. (1972b) were used to evaluate these storages. The number of larvae per 1000 m^3 were converted to milligrams of carbon per square metre using Chenoweth (1970) assuming an embayment depth of 40 m, a coastal depth of 60 m, and a carbon to dry weight ratio of 0.55. From these data, $S_1 = 2.2$ mg C·m^{-2}, $L_1 = 1.8$ mg C·m^{-2}, $S_3 = 0.024$ mg C·m^{-2}, and $L_3 = 0.91$ mg C·m^{-2}. From Graham (1982) and Graham et al. (1972a) we estimate that larval biomass per square metre of sea surface within the eastern embayments is about 10% of the coastal biomass in September; thus, $S_2 = 0.22$ mg C·m^{-2} and $L_2 = 0.18$ mg C·m^{-2}. Similarly, we assume that the western estuaries hold 10% of the larval biomass present in the western coastal waters, so $S_4 = 0.0024$ mg C·m^{-2} and $L_4 = 0.091$ mg C·m^{-2}.

LD is a storage of herring larvae in the western estuaries that have been marked for winter starvation. The initial value of LD is zero and larvae are marked for starvation when larval biomass exceeds 1.35 mg C·m^{-2} in the western estuaries on January 9. All larvae are marked for starvation within 7 d after which starvation begins.

Logic Programs

Starvation Program (SP) is shown as an energy circuit logic program in Fig. 4a. We chose 1965 as the base year to evaluate the parameters controlling the winter starvation mortality in the western estuaries because the spring larval cruise was early in this year, and therefore, inshore concentrations of herring larvae more accurately reflect the survival of the larvae overwintering in the estuaries rather than the inshore migration of larvae that overwintered in the coastal waters. The starvation mortality used in the model increased winter mortality of large larvae in the western estuaries in all years in which larval biomass (L_4) exceeded the carrying capacity threshold (ST) on a specified date (TS). An initial date (TS) of January 9 was chosen so that starvation began about a month after the fall die-off in years when the quantity of larvae in the estuaries exceeded (ST) the starvation threshold set at 1.35 mg C·m^{-2}. TD_2 is a week-long time delay before starvation begins (Blaxter 1962).

The logic program (LMF) describing fall large larval movements is shown in Fig. 4b. As long as the time (T) is less than the date when inshore movement stops (F) minus a 14-d lag (TD_1), large larvae move into the estuaries. Spring large larval movement is shown by logic program (LMS) (Fig. 4c). The spring inshore movement begins when the time (T) is later than March 1 (SP).

The logic program controlling the fall die-off ($LP_1 - LP_4$) is shown in Fig. 4d. Initially, ($T = 0$), autumn natural mortality is in effect. When the ratio of large larval biomass L_i to zooplankton biomass (Z_i) times ZFA_i exceeded the autumn support threshold (ZA_i), the fall die-off mortality is turned on (logic 1 to (S) on the flip-flop box) and autumn mortality is turned off (logic 1 to reset (R) on the other flip-flop box). When the ratio of larval biomass (L_i) to zooplankton biomass (Z_i) times ZFB_i is less than the winter support threshold (ZB_i) and the die-off mortality is on, the die-off mortality is turned off and winter mortality is turned on.

The transport program (TP) is shown as a flow chart in Fig. 4e. In this program, T is time, W_i is the time of a wind event, S1 is the quantity of small larvae in the retention area, and TS_1 is the quantity of small larvae subject to transport. This program is written in standard flow chart format and verbally explained in the text, so it will not be described in detail here.

Pathway Flows

k_{0i} is the amount of solar radiation absorbed by a square metre of sea surface. This value is calculated by taking the difference between the incident solar radiation J_{Ii} and the albedo J_{Ri}, estimated as 10% for latitude 44° (Von Arx 1962). J_{I3} and J_{I4} are 4720 cal·m^{-2}·d^{-1} (1 cal = 4.1868 J) and J_{R3} and J_{R4} are 472 cal·m^{-2}·d^{-1}, leaving 4248 cal·m^{-2}·d^{-1} to be absorbed by the western ecosystem. J_{I1} and J_{I2} are 4570 cal·m^{-2}·d^{-1} and J_{R1} and J_{R2} are 457 cal·m^{-2}·d^{-1}, leaving 4113 cal·m^{-2}·d^{-1} to be absorbed by the eastern ecosystems.

$k_{Ii}P_iJ_{Ri}$ is the phytoplankton gross primary production, GPP, for coastal and estuarine waters. From Garside et al. (1978) an average rate of 3 mg C·g Chl a^{-1}·h^{-1} primary production from summer to winter was calculated for the Sheepscot Estuary using the ^{14}C method. Assuming that this rate is typical of Sep-

tember primary production along the Maine coast and adding in 10% for phytoplankton respiration (Steemann Nielson and Hansen 1959), GPP is 464 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western coastal waters, 450 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuaries, 326 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coastal waters, and 268 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern embayments.

$k_{2i}P_i$ is the loss of phytoplankton biomass through respiration, sinking, and predation by animals other than zooplankton. This loss is taken as the difference between gross primary production and zooplankton grazing.

$k_3P_iZ_i$ is the phytoplankton grazed by zooplankton. We assume that microzooplankton graze 20% of their body weight per day and microzooplankton graze 60% of their weight per day (Parsons and Takahashi 1973, p. 116). Zooplankton grazing is 72.8 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western coast, 30 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuaries, 30 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coast, and 10 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern embayments.

$k_{4i}P_iZ_i$ is the food assimilated by zooplankton. The zooplankton assimilation rate is assumed to be 70% (Steele 1974).

$k_5Z_i(k_{4i}P_iZ_i)$ is the loss of zooplankton biomass through respiration (Ikeda 1970) and predation other than by herring larvae. It was calculated by difference.

$k_{6i}Z_iS_i$ is the amount of zooplankton biomass eaten by small herring larvae. Small herring larvae (11 mm average size) search about 1.5 $L \cdot h^{-1}$ (Blaxter and Hunter 1982) and have a capture rate assumed to average 50% over the interval from 7 to 15 mm. If these larvae feed only on microzooplankton during daylight hours, they can ingest a maximum of 0.105 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coastal area, 0.005 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern embayments, 0.0063 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western coastal area, and 0.0004 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuaries.

$k_{7i}Z_iL_i$ is the amount of zooplankton biomass eaten by large herring larvae. Large herring larvae (average size 17 mm in the east and 22 mm in the west) search an average of 7.5 $L \cdot h^{-1}$ and have a capture rate of 95% (Blaxter and Hunter 1982). Large larvae ingest 0.096 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coastal area, 0.005 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern embayments, 0.147 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coastal area, and 0.009 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuaries.

$k_{8i}Z_iS_i$ is the assimilation of small larvae. Blaxter and Hunter (1982) noted that growth averages 40% of ration and 45–51% of the energy assimilated. Thus, assimilation is between 78 and 90% of ingestion. We assume that assimilation is 90% for small larvae. Small larvae assimilate 0.095 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coastal area, 0.0045 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern embayments, 0.0057 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western coastal area, and 0.00035 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuaries.

$k_{9i}Z_iL_i$ is the assimilation of large herring larvae. Using an assimilation rate of 90%, assimilation is 0.087 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coastal area, 0.004 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern embayments, 0.132 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coastal area, and 0.008 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuaries.

$k_{10i}S_i$ is the respiration and natural mortality of small herring larvae. The respiratory rates for small larvae of size 0.15 mg were estimated from De Silva and Tytler (1973). Respiration of small larvae is 0.14 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coastal area, 0.014 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern embayments, 0.0015 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western coastal waters, and 0.00015 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuaries. Natural mortality of the small larvae in the eastern section was prorated by size of the larvae present. Larvae <10 mm in size were assigned a mortality of 29%·d^{-1} (Graham and Chenoweth 1973) and larvae between 10 and 15 mm were assigned a daily mortality of 5% (Oiested and Moksness 1981; Lough et al. 1985). Natural mortality is 0.16 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coastal section, 0.016 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern embayments, 0.006 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western coastal area, and 0.0006 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuaries.

$k_{11i}L_i$ is the respiration and natural mortality of the large larvae. Respiratory rates for large larvae of weight 1.1 mg were calculated from De Silva and Tytler (1973). The respiration of larvae is 0.08 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coastal waters, 0.008 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern embayments, 0.04 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western coastal waters, and 0.004 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuaries. An average natural mortality for the large larvae in fall was assumed to be 1%·d^{-1}. Graham and Davis (1971) measured natural mortalities from 1.5 to 3.5%·d^{-1} in early winter, and we assumed that large larval mortality during the milder fall period would be less than the smallest winter mortality. Natural mortality is 0.02 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern coast, 0.002 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern embayments, 0.01 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western coast, and 0.001 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuaries. Additional mortalities during the winter, $k_{11iW}L_i$, and for the fall die off, $k_{11iD}L_i$, were estimated from model simulations so that a good fit to the 1971 data was obtained. The starvation mortality, k_{114ST}, was calculated from model simulations to fit winter larval survival in 1965. Coefficient k_{114ST} is set so that all larvae are marked for starvation within 7 d. Coefficient k_D, the death rate from winter starvation, was set so that all larvae marked for starvation were dead within 30 d.

$k_{12}S_i$ is quantity of small herring larvae that become large larvae through growth. Assuming an average growth rate of 0.25 mm·d^{-1} (Graham and Townsend 1985), it will take about 16 d for the average small larva to become a large larva. Growth in the eastern coastal sector was assumed to be 0.35 mm·d^{-1} which is at the outer edge of the distribution observed by Graham and Townsend (1985). This change may not be unreasonable, since Graham and Townsend (1985) observed that growth rate increased as percent mortality increased and percent mortality was much higher in the east than in the west. We calculate growth rates of 0.19 mg $C \cdot m^{-2} \cdot d^{-1}$ for the eastern coastal area, 0.014 mg $C \cdot m^{-2} \cdot d^{-1}$ for the eastern embayments, 0.0015 mg $C \cdot m^{-2} \cdot d^{-1}$ for the western coastal area, and 0.00015 mg $C \cdot m^{-2} \cdot d^{-1}$ in the western estuarine area.

$k_{13i}J_W(S_{1,3} - S_{2,4})$ is the net transport of herring larvae shoreward by the wind. The net shoreward wind transport in August 1971 was 1.6×10^9 $m^3 \cdot d^{-1}$ which gives a transport of small larvae of 0.017 mg $C \cdot m^{-2} \cdot d^{-1}$ into the eastern embayments and 0.0002 mg $C \cdot m^{-2} \cdot d^{-1}$ into the western estuaries.

$k_{14i}J_T(S_{1,3} - S_{2,4})$ is the net transport of herring larvae shoreward by the tide. The tidal transport of larvae shoreward is 0.04 mg $C \cdot m^{-2} \cdot d^{-1}$ in the eastern section and 0.0004 mg $C \cdot m^{-2} \cdot d^{-1}$ in the west.

$k_{151,3}L_{1,3}$ is the fall migration of large larvae into the estuaries and embayments. Through model simulations using 1971 data we estimated that 0.043 mg $C \cdot m^{-2} \cdot d^{-1}$ of large larvae migrated inshore from the western coastal sector in fall 1971 and 0.033 mg $C \cdot m^{-2} \cdot d^{-1}$ moved into the eastern embayments. The spring migration, $k_{151S}L_{1,3}$ is allowed to vary from year to year in the western sectors to reflect the observed biomass distribution. In the eastern sectors the migration rate was set to reflect the 1973 springtime biomass distribution which was the only set of observations available for this area. Migration of

larvae from the coastal area into the estuaries and embayments results in an effective concentration of 1.76 times in the western area and 1.58 times in the eastern area due to the smaller area of the inshore sectors. The area concentration factor was also applied to larvae moved inshore by wind and tide.

$k_{16}S_1(J_C + J_{NE})$ is the loss of small larvae from the eastern coastal sector. It was calculated from the estimates of transport and larval concentrations and found to be 0.226 mg $C \cdot m^{-2} \cdot d^{-1}$.

$k_{17}S_1(J_C + J_{NE})$ is the small larval herring biomass which is transported into the western sector from the eastern sector and is calculated to be 0.143 mg $C \cdot m^{-2} \cdot d^{-1}$. The remainder, 0.083 mg $C \cdot m^{-2} \cdot d^{-1}$, is carried seaward between the sectors.

$k_{18}S_3(J_C + J_{NE})$ is the biomass of small larvae transported out of the western sector toward Massachusetts Bay. This number is calculated from the model to be 0.008 mg $C \cdot m^{-2} \cdot d^{-1}$.

Pigment Preservation in Lake Sediments: A Comparison of Sedimentary Environments in Trout Lake, Wisconsin

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Fluxes and concentrations of *a* phorbins and major algal carotenoids were quantified in sediment trap material and sediment cores from two basins of Trout Lake, Wisconsin (TrDH and TrAB). The basins were chosen to contrast the influence of oxygen content at the sediment–water interface (TrDH, oxic and TrAB, reducing), sediment accumulation rate, and focusing. Pigment diagenesis occurred in both basins, but transformations and destruction were more extensive in TrDH. Although untransformed chlorophyll *a* was the major phorbin deposited at the sediment surface of both basins (51–64 mol%), pigment destruction, coupled with transition to pheophytin, accounted for substantial losses, especially in oxic TrDH sediments. Fucoxanthin, peridinin, and diadinoxanthin, despite representing >70% of the deposited carotenoid flux, were substantially degraded or transformed in both basins. However, preservation was relatively high for secondary carotenoids, such as diatoxanthin and β-carotene, and for a major cryptomonad pigment, alloxanthin. Residual profiles in sediments show that pigment sedimentation from the epilimnion and accumulation in the permanent sediments are not directly related and that diagenesis must be considered in interpreting sedimentary pigments.

Les flux et les concentrations des phorbines *a* et des caroténoïdes principaux (algues) ont été quantifiés dans les matériaux formant des pièges à sédiments et dans les carottes sédimentaires en provenance de deux bassins de Trout Lake, dans le Wisconsin (TrDH et TrAB). Les bassins ont été choisis de façon à mettre en relief l'influence de la teneur en oxygène à l'interface eau–sédiment (TrDH oxique et TrAB réducteur), le taux d'accumulation des sédiments et la concentration. La diagénèse des pigments s'est produite dans les deux bassins, mais les transformations et la destruction étaient plus importantes dans le TrDH. Bien que la chlorophylle *a* non transformée ait constitué la principale phorbine déposée à la surface des sédiments des deux bassins (51 à 64 mol%), la destruction des pigments, associée à la transition à la phéophytines expliquait les pertes considérables, spécialement dans les sédiments du TrDH oxique. La fucoxanthine, la péridinine et la diadinoxanthine, même si elles représentaient moins de 70% du flux de caroténoïde déposé, étaient considérablement dégradées ou transformées dans les deux bassins. Cependant, la préservation était relativement élevée pour les caroténoïdes secondaires tels la diatoxanthine et le β-carotène, et pour un pigment cryptomonade important, l'alloxanthine. Les profils des résidus dans les sédiments montrent que la sédimentation des pigments de l'épilimnion et que l'accumulation dans les sédiments permanents ne sont pas directement reliées et que la diagénèse doit être envisagée dans l'interprétation relative aux pigments secondaires.

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Algal pigments are an important source of information on lake biogeochemistry. Chlorophyll *a* is a measure of primary productivity; carotenoids are biomarkers of algal taxa. Transformation products are indicative of trophic interactions and diagenetic processes. Pigment residues in sediments provide information on changes in productivity and trophic interactions. However, interpretation of algal pigments has been hampered by insufficient pigment-specific information on rates and products of degradation.

Several important transformations have been described. Conversion of chlorophyll to pheophorbide by metazoans has been widely used as a measure of grazing (Welschmeyer and Lorenzen 1985; Carpenter et al. 1986; Leavitt et al. 1989; Leavitt and Carpenter 1990b). Demetallation of chlorophyll to pheophytin occurs through biotic and abiotic processes (Keely and Brereton 1986). Similar to phorbin pathways, herbivores may enzymatically hydrolyze carotenoid esters such as fucoxanthin to their alcohol derivatives (Repeta and Gagosian 1984). Subsequent dehydration and epoxide opening to form dehydrates in sediments has been proposed (Repeta and Gagosian 1984). Conversion of carotenoid epoxides to loliolides in marine sediments during sulfate reduction has been reported, and conversion to carotanes during methanogenesis has been postulated (Repeta 1989). Other pathways lead to complete degradation or formation of unknown products of phorbins and carotenoids (Leavitt and Brown 1988; Hurley and Armstrong 1990; Leavitt and Carpenter 1990b). Photodegradation may be extensive within the photic zone (Carpenter et al. 1986). In sediments, phorbins associate with humic material and are degraded by microorganisms (Furlong and Carpenter 1988).

Information gained from sedimentary pigments has paralleled developments in analytical techniques. Vallentyne (1955, 1956) and Gorham (1960, 1961) related chlorophyll derivatives and carotenoids in sediments to lake trophic status using extrac-

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tion and spectrophotometric techniques. Subsequently, others (Guilizzoni et al. 1983; Swain 1985; Adams and Prentki 1986) have used sedimentary chlorophyll degradation products (SCDP) as a measure of eutrophy. Liquid–liquid extraction and column chromatography were used to separate carotenoids into hypophasic and epiphasic fractions (Gorham et al. 1974) and to isolate the blue-green algal pigments myxoxanthophyll and oscillaxanthin (Griffiths and Edmundson 1975; Griffiths 1978; Swain 1985). Paper chromatography (Daley et al. 1977), thin-layer chromatography (Zullig 1981, 1989), and high-performance liquid chromatography (HPLC) (Repeta 1982; Mantoura and Llewellyn 1983; Bidigare et al. 1985; Hurley 1988b) are now employed to identify individual phorbins and carotenoids and their derivatives.

The importance of algal pigments in paleolimnology is well recognized. Residual carotenoid stratigraphies have been related to changes in lake eutrophy associated with blue-greens and other algal classes (Griffiths and Edmundson 1975; Griffiths 1978; Zullig 1981, 1989; Swain 1985). Sedimentary phorbins and carotenoids in varved sediments of two manipulated lakes were shown to reflect cascading trophic interactions (Leavitt et al. 1989). Additionally, stratigraphies across a lake basin were used to assess sediment mixing and benthic processing and indicated that signal strengths of individual events are interpretable in moderately mixed sediments (Leavitt and Carpenter 1989).

Although sedimentary records have been used extensively, most evaluations have been qualitative because of limited information on pigment losses through degradation and influences of environmental conditions and processes. Recently, flux measurements have been used to quantify pigment production and loss. Transformation and degradation within the water column can be extensive (Repeta and Gagosian 1983, 1984, 1987; Welschmeyer and Lorenzen 1985; Carpenter et al. 1986, 1988; Furlong and Carpenter 1988; Leavitt et al. 1989; Hurley and Armstrong 1990). Phorbin losses through sedimentation, transformations, photodegradation, and other destructive pathways have been measured using a mass balance approach (Welschmeyer and Lorenzen 1985; Carpenter et al. 1986, 1988). Similarly, Repeta and Gagosian (1984, 1987) assessed water column processes affecting carotenoid degradation.

Flux estimates based on sediment traps and dated sediment profiles have also been used to estimate pigment degradation during the early stages of sedimentation. Furlong and Carpenter (1988) reported >99% degradation of the pheopigment flux leaving the photic zone in Dabob Bay prior to incorporation into surface sediments. In more productive Saanich Inlet and Lake Washington, degradation represented about 90–99 and 96%, respectively, of the sediment trap pigment flux (Furlong 1986). Sediments of Saanich Inlet and Lake Washington are predominantly anoxic, suggesting that pigment preservation is increased under anoxic conditions (Furlong and Carpenter 1988). In Lake Mendota, a seasonally anoxic lake with a mass sediment accumulation rate twice that of Dabob Bay, we estimated that >65% of the phorbin and >70% of the carotenoid fluxes to hypolimnetic sediment traps were lost during incorporation into surface sediments (Hurley and Armstrong 1990). Further loss occurred during burial of surface sediment (Hurley 1988a).

Information on sedimentary conditions influencing degradation is limited. Factors believed to enhance preservation include low oxygen concentration, temperature, light penetration and sediment resuspension, high sedimentation rates,

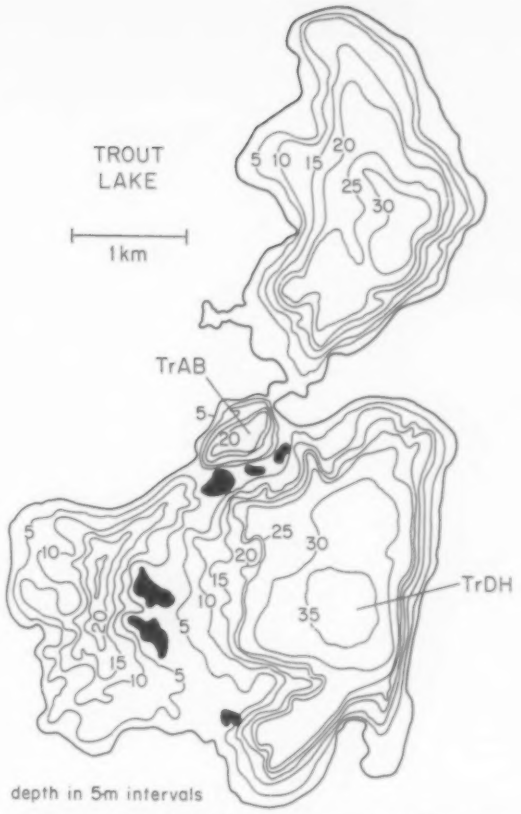

FIG. 1. Map of Trout Lake, Wisconsin. Blackened areas represent islands.

absence of benthic organisms in sediments, and increased levels of eutrophy (Sanger 1988). Incorporation of conditional rate constants reflecting these factors into diagenetic models (Berner 1980) would enhance interpretation of sedimentary pigment records.

In this study, we evaluated pigment degradation in two sedimentary basins of Trout Lake, Wisconsin. Among the individual basins of this multibasin lake are sediment depositional zones with contrasting physical and chemical characteristics (Robertson 1984; Robertson and Ragotzkie 1990). In particular, two basins offer the ability to compare effects of sedimentation rate, sediment focusing, and seasonal sediment anoxia on pigment preservation. We report pigment-specific degradation rates of phorbins and carotenoids in surficial sediments, based on sediment-trap measured fluxes from the water column and accumulation rates in surficial sediments, and assess resulting sedimentary profiles.

Materials and Methods

Study Site

Trout Lake is a dimictic, softwater lake situated in noncalcareous glacial outwash in the Northern Highlands State Forest District in Vilas County, Wisconsin (46°20′N, 89°41′W). Details on chemical, physical, and biological parameters are available from the Long Term Ecological Research – Northern Temperate Lakes project (Magnuson et al.

1984; LTER 1987). In addition to its location as the terminus of the local groundwater flow system (Kenoyer 1986; Krabbenhoft and Anderson 1986), Trout Lake receives inflow from three streams and has one outflow stream.

An important characteristic of Trout Lake is its multibasin morphometry (Robertson 1984) (Fig. 1). The basin located in the northern part of south Trout Lake (TrAB) is 20 m deep, surrounded by islands and rock bars, and exhibits strong hypolimnetic oxygen depletion below the thermocline during summer. In contrast, the large southeastern basin in south Trout Lake (TrDH) is 35 m deep and contains a well-oxygenated hypolimnion that exhibits oxygen depletion in late summer only 1–4 m above the sediment surface (LTER 1987).

Field Sampling

Particulate matter that settles to the sediment surface was collected by sediment traps. Acrylic traps, 75 cm tall (4:1 aspect ratio over collection area, funneled to a 250-mL collection bottle), were designed for optimal collection efficiency (Gardner 1980; Shafer 1988). Duplicate traps, poisoned with sodium azide, were positioned in the hypolimnion of each basin. The depth of trap deployment was 17 m (3 m above the sediment surface) in TrAB and 21 m (12 m above the sediment) in TrDH. Sampling intervals were approximately 28 d.

For pigment extraction, amounts in the range of 5–50 mg dry weight were collected on Whatman GF/F filters. Wet filters were frozen and stored at −10°C. Pigment extraction from filters was performed within 3 d. Mass flux of each fraction was determined by filtering aliquots through preweighed 0.4-μm Nuclepore filters. The remainder of the material was centrifuged, freeze-dried, and stored for various elemental analyses.

Sediment cores were taken from TrDH and TrAB using a frozen crust coring apparatus (Swain 1978). Visual inspection revealed differences in physical characteristics between basins. TrAB contained compact sediments, with incomplete laminations, while the sediments of TrDH were flocculent (Hurley 1988a). Benthic activity (i.e. the presence of chironomid tubes) was clearly evident in TrDH sediments, and intact chironomid tubes in the upper 5 cm of sediments confirmed the integrity of the frozen cores. Cores, measuring approximately 1 m, were sectioned in 0.5- to 1-cm intervals at the top of the core (upper 5–10 cm) and into 5- and 10-cm intervals in mid- and deeper core sections. Due to the compactness of TrAB cores, more detailed samples were taken in the upper 10 cm. Sediment samples were taken from the outer rim of the frozen crust to avoid possible effects of smearing associated with insertion of the core barrel into sediments.

Samples for pigment analysis were placed in Teflon-sealed vials, evacuated under nitrogen to prevent oxidation, and stored at −10°C. Approximately 1 g of wet sediment was extracted for pigment analysis. The amount of sediment on a dry weight basis was calculated from water content measurement on separate subsamples. Due to the water content of the sediments (85–99%), the 1-g wet samples represented about 5–50 mg of dry sediment. After replicates were taken on appropriate samples, the remainder was freeze-dried for elemental analysis.

Laboratory Analyses

Techniques for sample preparation, pigment extraction, sediment preparation, internal standard addition, and quantification were described by Hurley (1988a). Samples were handled under subdued lighting and in a black-walled hood during concentration steps. To minimize exposure to oxygen, samples and standards were purged with nitrogen and sealed under Teflon-lined caps before prolonged periods of storage.

Based on comparisons of extraction solvents (Hurley 1988a; Bowles et al. 1985; Mantoura and Llewellyn 1983), 90% acetone was used for extraction. For samples that contained significant amounts of water (sediment trap material, sediments), the amount of acetone added was adjusted so that the final extract contained 90% acetone. Exhaustive sonicated extraction revealed insignificant pigment release from sediments after three extractions. Extracts were filtered through 25-mm Nylon 66, 0.2-μm filters (Alltech Assoc.) and then reduced to about 4 mL using rotary evaporation. Ion pairing agent (0.5 mL of 0.05 M PIC A, tetrabutyl ammonium phosphate, Waters Assoc.), necessary for separation of polar chlorophyll derivatives, was added to each sample, and the final solution was brought to 5.0 mL using acetone.

Pigment measurements were performed using the following instrumentation: Waters model 600 solvent delivery system, WISP 712A autosampler with refrigeration unit (−4°C), Waters 481 absorbance detector (set at 440 nm), Waters 420C fluorescence detector (excitation wavelength = 420 nm; emission wavelengths >650 nm), and Waters 820 data system. Separations were performed on a 15-cm Waters NOVA-PAK C-18 column, protected by a C-18 guard column. The solvent system, a modification of the system described by Mantoura and Llewellyn (1983), consisted of an initial solvent of 80% methanol and 20% 0.005 M PIC A with a 20-min linear gradient to 80% methanol and 20% acetone (Hurley 1988b). Final conditions were held for 15 min before ramping to original conditions and column reequilibration. Flow rate was held constant throughout the run at 1.5 $mL \cdot min^{-1}$.

Chlorophyll *a* and β-carotene were obtained from Sigma Co. Chlorophyll *a* derivatives were prepared by oxidation (allomers), acidification (pheophytin), and dephytolization (chlorophyllide and pheophorbide) (Jeffrey 1968; Brown 1968; Barrett and Jeffrey 1971; Sartory 1985). Standards of lutein + zeaxanthin, fucoxanthin, and echinenone were donated by Hoffmann-LaRoche of Basle, Switzerland. Carotenoids which were not commercially available were extracted from algal cultures of known pigment composition (Hertzberg et al. 1971; Johansen et al. 1974; Mantoura and Llewellyn 1983). Retention indices, published absorbance maxima, and extinction coefficients (Brown 1968; Davies 1976; Mantoura and Llewellyn 1983; Lorenzen and Downs 1986) were used for identification and quantification. Spectral scans of individual components were conducted with a Varian DMS-80 UV-VIS spectrophotometer or in-line on HPLC with a Waters 990 diode array detector.

Sediment accumulation rates and surface mixed layer (SML) depths were determined from unsupported ^{210}Pb (Robbins and Edgington 1975; Robbins et al. 1977) with modifications by Talbot (1981). Total carbon was determined on freeze-dried material using the dry combustion method of the Perkin-Elmer model 240C elemental analyzer (Fullerton 1988).

Pigment concentrations are expressed as micromoles per gram of particulate matter on a dry weight basis, micromoles per gram of carbon, and mole percent, e.g. mole percent carotenoid i = (moles carotenoid i/moles total carotenoid) × 100.

Results

Physical and Chemical Characteristics of the Water Column

Development of thermal stratification, combined with influences of basin shape and hypolimnion volume, resulted in

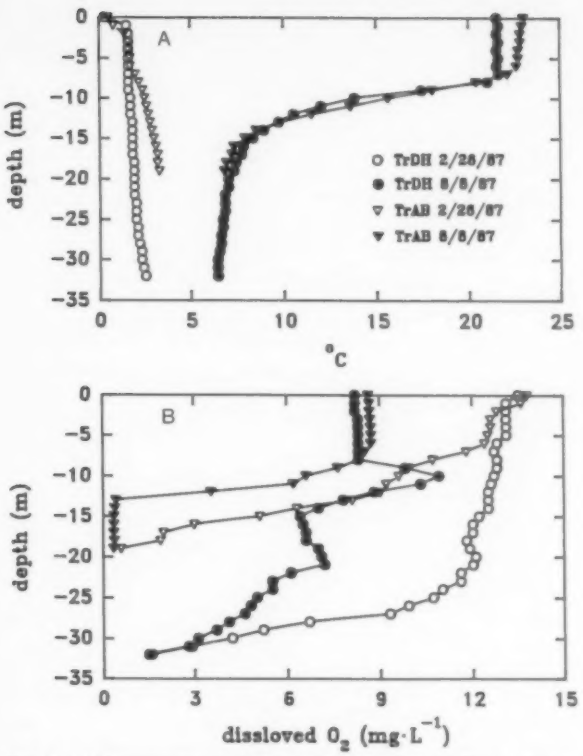

FIG. 2. (A) Temperature and (B) dissolved oxygen profiles in the two basins of Trout Lake.

substantial differences in oxygen depletion between basins, as observed in other lakes (Charlton 1980). In Trout Lake, thermocline depth during maximum stratification is similar in the two basins (Fig. 2A), but oxygen content is substantially lower in the hypolimnion of TrAB (Fig. 2B). Similar data from Robertson (1984) show oxygen depletion in the bottom 2 m of TrAB by early May 1982, suggesting either rapid depletion during oxidation of organic matter from the spring diatom bloom or incomplete spring mixing of the water column in this sheltered basin. Although TrAB becomes fully mixed at fall overturn, oxygen is depleted to <1 mg·L^{-1} near the sediment surface in winter (Fig. 2B). In contrast, oxygen is present in the hypolimnion of TrDH, reaching maximum levels at the base of the thermocline and dropping below 3 mg·L^{-1} only within 1 m above the sediment surface, both during maximum stratification and under ice-cover.

Levels of redox-controlled chemical constituents reflect differences between basins in oxygen depletion (LTER 1987). In particular, sulfate is depleted in the hypolimnion of TrAB relative to TrDH. Similarly, greater loss of sulfate occurs in sediment porewaters of TrAB (A. Giblin, The Ecosystems Center, Marine Biological Station, Woods Hole, MA, pers. comm.).

Fluxes to Sediments

Mass and carbon deposition

Sediment traps deployed in the hypolimnion of each basin provided data on mass and pigment fluxes to the sediment surface during ice-free periods (Fig. 3). Depositional fluxes were measured over several periods of approximately 28 d each from

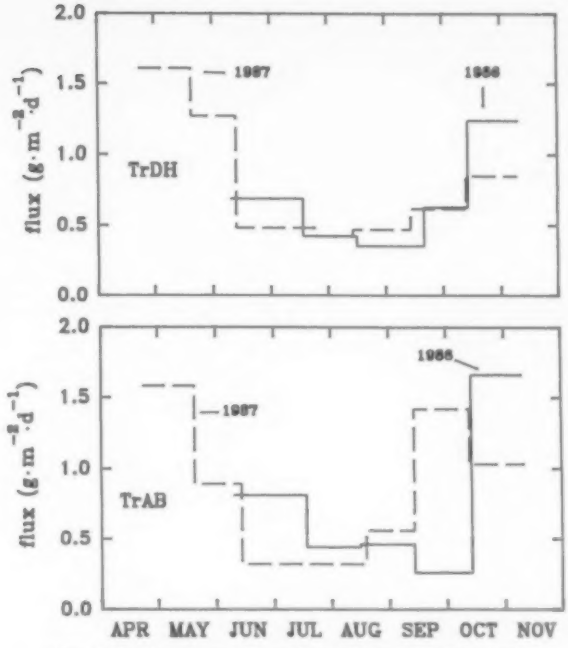

FIG. 3. Sediment trap fluxes in TrDH and TrAB basins.

June to November 1986 and April to November 1987. Total fluxes collected differed between years, since the April–May period was not sampled in 1986. Thus, 1986 data reflect absence of the important spring diatom depositional event. For comparable time periods during 1986 and 1987, however, depositional patterns were similar (Fig. 3). The lowest deposition rates occurred in midsummer in both basins during 1986 and 1987.

Total flux measurements may be biased low. Trap removal in early November, approximately 6–8 wk prior to ice-cover, may have excluded collection of a portion of the fall diatom bloom. For example, in Lake Mendota, the highest pigment fluxes occurred in late November (Hurley and Armstrong 1990). Also, production occurring under ice was not sampled, since traps were not deployed during ice-cover.

Trout Lake, a typical dimictic lake, exhibits strong phytoplankton blooms after spring and fall mixing. Diatoms are dominant during spring and fall events, as reflected by particulate biogenic silica deposition (Hurley 1984). Mass deposition rates during the spring bloom in 1987 were similar between basins (Fig. 3). Fluxes associated with the fall bloom in TrDH were slightly greater in 1986 than in 1987. In TrAB, fall mixing of nutrient-rich hypolimnetic water and the subsequent diatom bloom may have occurred about 1 mo earlier in 1987 than in 1986, as reflected by mass deposition rates.

Deposition rates of both mass and total carbon for given sampling intervals were similar between basins (Table 1). Although total deposition was greater in 1987, due to a longer trapping period, mean rates of organic carbon deposition ranged only between 0.15 and 0.18 g·m^{-2}·d^{-1}.

Phorbin sedimentation

Fluxes of major phorbins (chlorophyll *a*, chlorophyllide *a*, pheophytin *a*, and pheophorbide *a*) to surface sediments were calculated from analyses of sediment trap material (Table 2). Over the study period, untransformed chlorophyll *a* was the

TABLE 1. Annual ($g \cdot m^{-2}$) and mean ($g \cdot m^{-2} \cdot d^{-1}$) depositional fluxes in the two lake basins.

Basin and trap period	Mass	Organic C
TrDH		
8 June 1986 – 4 Nov. 1986		
Total flux	95.6	21.7
Mean flux rate	0.65	0.15
22 Apr. 1987 – 3 Nov. 1987		
Total flux	154.6	30.7
Mean flux rate	0.79	0.16
TrAB		
8 June 1986 – 4 Nov. 1986		
Total flux	105.0	25.6
Mean flux rate	0.71	0.17
22 Apr. 1987 – 5 Nov. 1987		
Total flux	168.4	35.3
Mean flux rate	0.85	0.18

TABLE 2. Phorbin flux to the sediment surface of Trout Lake measured using sediment traps. The 1986 flux is from 8 June to 4 August; the 1987 flux is from 22 April to 3 November.

	TrDH		TrAB	
	Flux ($\mu mol \cdot m^{-2}$)	Composition (mol%)	Flux ($\mu mol \cdot m^{-2}$)	Composition (mol%)
Chlorophyll *a*				
1986	31.4	61	42.1	64
1987	60.6	55	57.2	51
Pheophorbide *a*				
1986	7.6	15	8.4	13
1987	28.7	26	32.5	29
Pheophytin *a*				
1986	8.8	17	10.5	16
1987	9.6	9	9.0	8
Chlorophyllide *a*				
1986	3.2	6	4.9	7
1987	12.4	11	12.5	11

most abundant phorbin delivered to sediment traps (51–64 mol%). Deposition of pheophorbide *a*, an indicator of heterotrophy by metazoans, represented only 13–29% of the phorbin flux. Chlorophyllide *a*, abundant in both rapidly growing and senescent diatoms (Jeffrey and Hallegraeff 1987), together with pheophytin *a*, represented the remainder of the phorbins (19–23%).

Annual fluxes for 1986 and 1987 reflect different periods of sediment trap deployment. During early 1987, deposited phorbins in both basins (22 April 1987 to 11 June 1987) contained 45–57 mol% pheophorbide (Hurley 1988a), indicative of grazing by zooplankton. However, since senescent diatoms were abundant in sediment traps, some formation of pheophorbide through demetallation of chlorophyllide also may have occurred. Traps in 1986 were installed after this event; thus, the 1986 flux was dominated more strongly by chlorophyll deposition.

Carotenoid sedimentation

As organic matter deposition in Trout Lake is dominated by siliceous algae (Hurley 1984; LTER 1987), periods of high deposition rates (Fig. 3) reflect diatom blooms occurring after ice-out and after fall mixing. Thus, three major carotenoids common to diatoms (fucoxanthin, diadinoxanthin, and diatoxanthin) constituted 70–80% of the total carotenoid flux to sediment traps (Table 3). Additional carotenoids included β-carotene, those indicative of green and blue-green algae (lutein and zeaxanthin), cryptomonads (alloxanthin), and dinoflagellates (peridinin and diadinoxanthin). Sedimentation of green and blue-green algae could not be distinguished because lutein and zeaxanthin were not separated from each other. Although total carotenoid fluxes differed between 1986 and 1987 due to different trapping periods, molar composition was similar (Table 3), confirming the importance of Chrysophyta (mainly diatoms) in depositional fluxes throughout the ice-free period.

Surficial Sediment Pigments

Comparisons of trap material and surface sediments indicate that substantial alteration of pigments has occurred during initial stages of incorporation (Table 4). Additionally, surficial sediment pigments differ appreciably between basins. In TrAB, chlorophyll *a* is the dominant phorbin in trap material (51 mol%) and surface sediments (50 mol%). However, pheophytin content increases from 8 mol% in trap material to 25 mol% in surface sediment, most likely through losses of chlorophyllide and pheophorbide. In TrDH, pheophytin *a* is the dominant sediment phorbin, increasing from 9 mol% in traps to 49 mol% in sediments and suggesting demetallation of chlorophyll *a* as a major diagenetic process. Chlorophyllide is reduced from 11 to 0.4 mol% upon sedimentation, suggesting either complete degradation or a similar demetallation of chlorophyllide as a source of pheophorbide.

Six major carotenoids in Trout Lake sediments (fucoxanthin, alloxanthin, diatoxanthin, lutein + zeaxanthin, and β-carotene) were identified by HPLC, confirmed by absorbance spectra and quantified. Diadinoxanthin and peridinin, although present in sediment trap material, were not observed in significant amounts in the sediments. Several other smaller peaks were also present but have not been identified. The discussion is limited to the five largest carotenoid chromatographic peaks. "Total carotenoid" content and "mole percent" are based solely on the sum of these six major carotenoids.

Carotenoid composition also suggests major diagenetic changes within surficial sediments in both basins. Two carotenoids present in appreciable concentrations in sediment traps (peridinin and diadinoxanthin) were not observed in quantifiable amounts in surface sediments. Fucoxanthin, the dominant carotenoid present in sediment traps of both basins (55 mol%), was substantially depleted in surface sediments of both basins, accounting for only 18 and 8 mol% in TrAB and TrDH, respectively. However, minor carotenoids in sediment trap material (diatoxanthin, alloxanthin, lutein + zeaxanthin, and β-carotene) were dominant carotenoids in surface sediments.

Comparisons of molar distributions show that major alterations of pigments occur during the early stages of diagenesis. Pigment deposition and accumulation fluxes are compared below to obtain quantitative estimates of parent pigment loss.

Sediment Mass Accumulation Rates

Sedimentation rates, based on ^{210}Pb profiles (Fig. 4), differed dramatically between basins. The disparity between the

TABLE 3. Major carotenoid pigments deposited at the sediment surface. The 1986 flux is from 8 June to 4 November. The 1987 flux is from 22 April to 3 November. ND = not detected.

	TrDH		TrAB	
	Flux (μmol·m^{-2})	Composition (mol%)	Flux (μmol·m^{-2})	Composition (mol%)
Fucoxanthin				
1986	13.0	57	19.6	62
1987	31.4	59	29.9	58
Diadinoxanthin				
1986	2.20	10	1.45	5
1987	5.25	10	5.89	11
Diatoxanthin				
1986	2.37	10	4.91	15
1987	9.56	9	9.00	8
Alloxanthin				
1986	2.90	13	2.45	8
1987	3.97	7	3.49	7
Lutein + zeaxanthin				
1986	1.76	8	2.46	7
1987	3.35	6	2.26	4
β-Carotene				
1986	0.58	3	0.80	3
1987	1.67	3	2.31	4
Peridinin				
1986	ND	0	ND	0
1987	1.83	3	1.05	2

two profiles corresponds to a calculated mass accumulation rate that is five times greater in TrAB than in TrDH (Table 5). A SML exists in both basins. In TrAB, ^{210}Pb profiles revealed a mixed depth of about 7 cm (0.39 g·cm^{-2}). The lack of visible benthic organism activity in TrAB suggests that the mixing is mediated by physical rather than biological processes. Chironomid activity was clearly evident in TrDH sediments, yet ^{210}Pb profiles showed a similar mixed depth of 7 cm, corresponding to a mass depth of 0.26 g·cm^{-2}. Coupled with the lower sedimentation rate, however, the residence time of particles in the mixed layer of TrDH is 32 yr as compared with only 5.4 yr in TrAB. Thus, particles are subjected to SML conditions (benthic processing and oxic conditions) about six times longer in TrDH than in TrAB.

When comparing sedimentation rates calculated from sediment traps with those of sediment cores (accumulation), effects of sediment focusing must be considered. The rain of particulate matter from the epilimnion (measured by sediment traps) may be focused into a smaller depositional zone. Also, postdepositional migration at the sediment surface may move material to deep, low-energy regions, resulting in higher sedimentation rates at these depths.

The overall focusing correction factor F_{TOT} is

$$(1) \quad F_{TOT} = F_{MOR} \cdot F_{PDM}$$

where F_{MOR} is the morphometric factor and F_{PDM} is the factor attributable to postdepositional migration; F_{MOR} is calculated as the ratio of the surface area of the sediment depositional zone to the mean surface area of the midpoint of the epilimnion (where most phytoplankton growth is assumed to occur in these lakes). In Trout Lake, the epilimnion is about 12 m deep and the sediment depositional zone begins near 15 m. Therefore:

$$(2) \quad F_{MOR} = \frac{\text{surface area of 15 m stratum}}{\text{surface area of 6 m stratum}}$$

Surface areas for Equation 2 were taken from Robertson (1988), resulting in values of F_{MOR} = 0.61 and 0.69 for TrAB and TrDH, respectively.

Although we cannot evaluate F_{PDM} directly, accumulation of ^{210}Pb is used to measure relative focusing between the two basins (Fig. 4). Estimation of ^{210}Pb accumulated in each basin over three half-lives (67 yr) revealed that almost four times as much ^{210}Pb has accumulated at the coring site (40.8 pCi·cm^{-2}; 1 pCi = 37 mBq) in TrAB as compared with TrDH (10.5 pCi·cm^{-2}). Apparently, postdepositional migration of surface sediment strongly focuses material in TrAB relative to TrDH. The resulting focusing correction factor for TrAB relative to TrDH is 0.26.

Focusing can also be assessed by comparing the flux of ^{210}Pb to the lake surface with the accumulation flux in surface sediments. Talbot and Andren (1983) calculated the atmospheric flux to be 0.7 pCi·cm^{-2}·yr^{-1} in the Trout Lake region, while Binford and Brenner (1986) estimated 0.5 pCi·cm^{-2}·yr^{-1} to represent a global average. Extrapolated surface sediment ^{210}Pb concentrations, coupled with sedimentation rates, yield sediment ^{210}Pb accumulation rates of 2.37 and 0.53 pCi·cm^{-2}·yr^{-1} for TrAB and TrDH, respectively. Thus, comparisons of atmospheric to sediment fluxes indicate that sediment focusing is minimal in TrDH and substantial in TrAB. Estimated surface concentrations of ^{210}Pb are about 40% lower than in nearby Crystal Lake (Talbot 1981; Talbot and Andren 1984), suggesting that our estimates of sedimentation flux may also be low. Due to a relatively thick SML, estimated ^{210}Pb concentrations (unmixed) are uncertain by as much as 20%.

We have used basin morphometry and ^{210}Pb data to estimate minimum and maximum focusing correction factors. Using F_{PDM} = 1 in TrDH and the above values for F_{MOR}, we obtain maximum correction factors of 0.69 for TrDH and 0.16 for

TABLE 4. Comparison of molar composition of pigments in sediment traps and surface sediments. Concentration comparison is based on concentration (μmol·g sediment^{-1} or particulate matter) normalized to total carbon.

	Trap (mol%)	Sediment (mol%)	Concentration ratio of sediment to trap
	TrAB		
Phorbins			
Chlorophyll *a*	51	50	1.95
Pheophytin *a*	8	25	5.67
Chl *a* + phytin *a*	59	75	3.18
Pheophorbide *a*	29	18	0.61
Chlorophyllide *a*	11	6	0.96
Carotenoids			
Fucoxanthin	55	18	1.09
Diadinoxanthin	11	0	—
Peridinin	2	0	—
Diatoxanthin	17	34	5.7
Alloxanthin	6	19	8.5
Lutein + zeaxanthin	4	12	9.2
β-Carotene	4	16	12.5
	TrDH		
Phorbins			
Chlorophyll *a*	55	19	0.29
Pheophytin *a*	9	49	2.39
Chl *a* + phytin *a*	64	68	0.58
Pheophorbide *a*	26	35	0.70
Chlorophyllide *a*	11	0.4	0.02
Carotenoids			
Fucoxanthin	55	8	0.41
Diadinoxanthin	9	0	—
Peridinin	3	0	—
Diatoxanthin	16	37	0.82
Alloxanthin	7	23	2.01
Lutein + zeaxanthin	6	17	4.27
β-Carotene	3	16	9.30

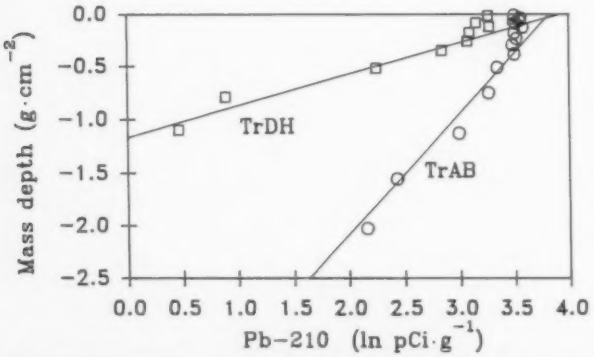

FIG. 4. ^{210}Pb content of TrAB and TrDH cores. Regression lines used to calculate sedimentation rate are indicated. 1 pCi = 37 mBq.

TrAB. If we assume no focusing in TrDH, as suggested by atmospheric to sediment ^{210}Pb comparisons, we obtain minimum correction factors of 1.0 in TrDH and 0.26 in TrAB. In contrast with measured mass sedimentation rates (Table 5), minimum to maximum ranges of focusing-corrected rates (grams per square centimetre per year) are 64–104 for TrAB and 55–80 for TrDH.

TABLE 5. Mass and linear sedimentation rates, SML depths, and residence time of particles in SML, based on ^{210}Pb activity in cores.

	Sedimentation rate		SML		
	Mass ($g \cdot m^{-2} \cdot yr^{-1}$)	Linear ($cm \cdot yr^{-1}$)	Mixed depth (cm)	Mass depth ($mg \cdot cm^{-2}$)	Residence time (yr)
TrAB	403 ± 51	0.66 ± 0.14	7	0.39	5.4
TrDH	80.1 ± 11.3	0.18 ± 0.02	7	0.26	32

Pigment Degradation in Surface Sediments

Pigment deposition to surface sediments (P_{in}, micromoles per square metre per year) is calculated as

$$(3) \quad P_{in} = \sum_i w_d C_d$$

where w_d is the particulate matter deposition rate from the water column (grams per square metre per year) and C_d is the pigment concentration of the trap-collected particulate matter (micromoles per gram) for each individual trap period, i. Similarly, removal of particulate-associated pigment from the SML by burial (P_{out}, micromoles per square metre per year) can be calculated by

$$(4) \quad P_{out} = w_s^* C_{SML}$$

where C_{SML} is the pigment concentration in the SML (micromoles per gram) and w_s^* (grams per square metre per year) is the focus-corrected mass accumulation rate of sediments, calculated from the measured rate (w_s) from ^{210}Pb profiles:

$$(5) \quad w_s^* = w_s F_{TOT}$$

Fluxes (micromoles per square metre per year) of constituents measured with sediment traps can be compared with the focus-corrected fluxes measured in the SML. Pigment input to the SML occurs through deposition of particles from the water column, and loss occurs through diagenesis and burial below the mixed layer:

$$(6) \quad \frac{d(C_{SML})(Z_{SML})D_B}{dt} = P_{in} - P_{out} - P_{dg}$$

where D_B is the sediment bulk density (grams per cubic metre) and Z_{SML} is SML thickness (metres). For steady-state conditions, C_{SML} and Z_{SML} are assumed to be constant on an annual average basis, and pigment removal by diagenesis (P_{dg}, micromoles per square metre per year) at the sediment surface is calculated as

$$(7) \quad P_{dg} = P_{in} - P_{out}$$

and

$$(8) \quad \%\,\text{deg} = \frac{P_{in} - P_{out}}{P_{in}} \times 100$$

where % deg is the percentage of sedimented pigment degraded. Results of this comparison are presented in Table 6. We note that trap placement may have allowed some below-trap pigment losses within the water column, especially in TrDH. Trap to

TABLE 6. Pigment fluxes in surface sediments based on mass fluxes and pigment concentrations for sediment trap material and surface sediments for the three lake basins studied. Values are in $\mu mol \cdot m^{-2} \cdot yr^{-1}$ for all pigment fluxes. Carbon flux is $g \cdot m^{-2} \cdot yr^{-1}$. % deg is the percent removed by diagenesis. Values of P_{out} have been corrected for focusing using Equation 3.

	P_{in}	Minimum P_{out}	Maximum P_{out}	Minimum P_{dg} (%)	Maximum P_{dg} (%)
			TrAB		
Total C	35.2	10.5	17.1	52	70
Total phorbin	111.4	69.4	112.8	−1	38
Chlorophyll *a*	57.4	34.7	56.4	1	39
Pheophytin *a*	9.0	17.6	28.6	−217	−95
Chl *a* + phytin *a*	66.2	52.4	85.2	−29	21
Pheophorbide *a*	32.5	12.5	20.3	38	61
Chlorophyllide *a*	12.5	4.5	7.31	42	64
Total carotenoid	53.9	48.7	79.1	−47	10
Fucoxanthin	29.9	9.00	14.2	51	70
Diadinoxanthin	5.89	0	0	100	100
Peridinin	1.05	0	0	100	100
Diatoxanthin	9.00	16.4	26.6	−196	−82
Alloxanthin	3.49	9.29	15.1	−332	−166
Lutein + zeaxanthin	2.30	6.03	9.80	−326	−162
β-Carotene	2.31	7.98	13.0	−461	−245
			TrDH		
Total C	30.7	10.1	14.6	52	67
Total phorbin	109.5	21.8	31.6	71	80
Chlorophyll *a*	60.6	4.16	6.03	90	93
Pheophytin *a*	9.60	10.7	15.5	−61	−11
Chl *a* + phytin *a*	70.2	14.9	21.6	69	79
Pheophorbide *a*	28.7	7.69	11.1	61	73
Chlorophyllide *a*	12.4	0.09	0.13	99	99
Total carotenoid	57.1	21.5	31.2	45	62
Fucoxanthin	31.4	1.64	2.38	92	95
Diadinoxanthin	5.25	0	0	100	100
Peridinin	1.83	0	0	100	100
Diatoxanthin	9.56	7.89	11.4	−19	17
Alloxanthin	3.97	4.92	7.13	−79	−24
Lutein + zeaxanthin	3.35	3.66	5.30	−58	−9
β-Carotene	1.67	3.35	4.86	−190	−100

sediment distances were 12 m (oxic) in TrDH and 3 m (anoxic) in TrDH. Below trap losses would be attributed to degradation at the sediment surface. Also, diagenesis occurring during post-depositional focusing is included within diagenesis in surface sediment.

Flux estimates for total carbon reveal similar rates of diagenesis between basins. Although total organic carbon may not reflect specific organic constituents, bulk carbon remineralization is slightly higher in TrAB.

Total phorbin degradation is substantially greater in TrDH. Approximately 70–80% of the delivered flux is degraded in surface sediments of TrDH, as compared with less than 38% in TrAB. The greatest flux difference between basins for individual phorbins was for chlorophyll *a*, accounting for about 50% of the total phorbin degradation in TrDH (>90% of chlorophyll) and <20% in TrAB (<39% of chlorophyll). Demetallation of chlorophyll *a* to pheophytin *a* may account for some of the chlorophyll loss. However, when summed, the effects of demetallation are negated and also show greater preservation of phytollated derivatives in TrAB (<21% degraded) than in TrDH (>69% degraded). In TrAB, substantial differences are observed in preservation of phytol- and nonphytol-containing derivatives. Pheophorbide (38–61%) and chlorophyllide (42–64%) degradation exceeds that of phytollated derivatives (<21%). In TrDH sediments, however, chlorophyllide is nearly completely degraded (99%), and loss of pheophorbide (61–73%) is similar to that of phytol-containing derivatives (69–79%). In both basins, demetallation of chlorophyllide may account for some pheophorbide gain.

Total carotenoid degradation also differed substantially between basins. In TrAB, degradation amounted to <10% of the summed eight major carotenoids quantified, compared with 45–62% in TrDH. Loss of total carotenoids in both basins resulted mainly from the degradation of diadinoxanthin, peridinin (both absent in sediments), and fucoxanthin. In TrDH, >92% of the deposited fucoxanthin was lost, yet only 51–70% of this most abundant carotenoid in deposited material was lost in TrAB. In contrast with these epoxide-containing carotenoids, diol-containing carotenoids (diatoxanthin, alloxanthin, and lutein + zeaxanthin) together with β-carotene are well pre-

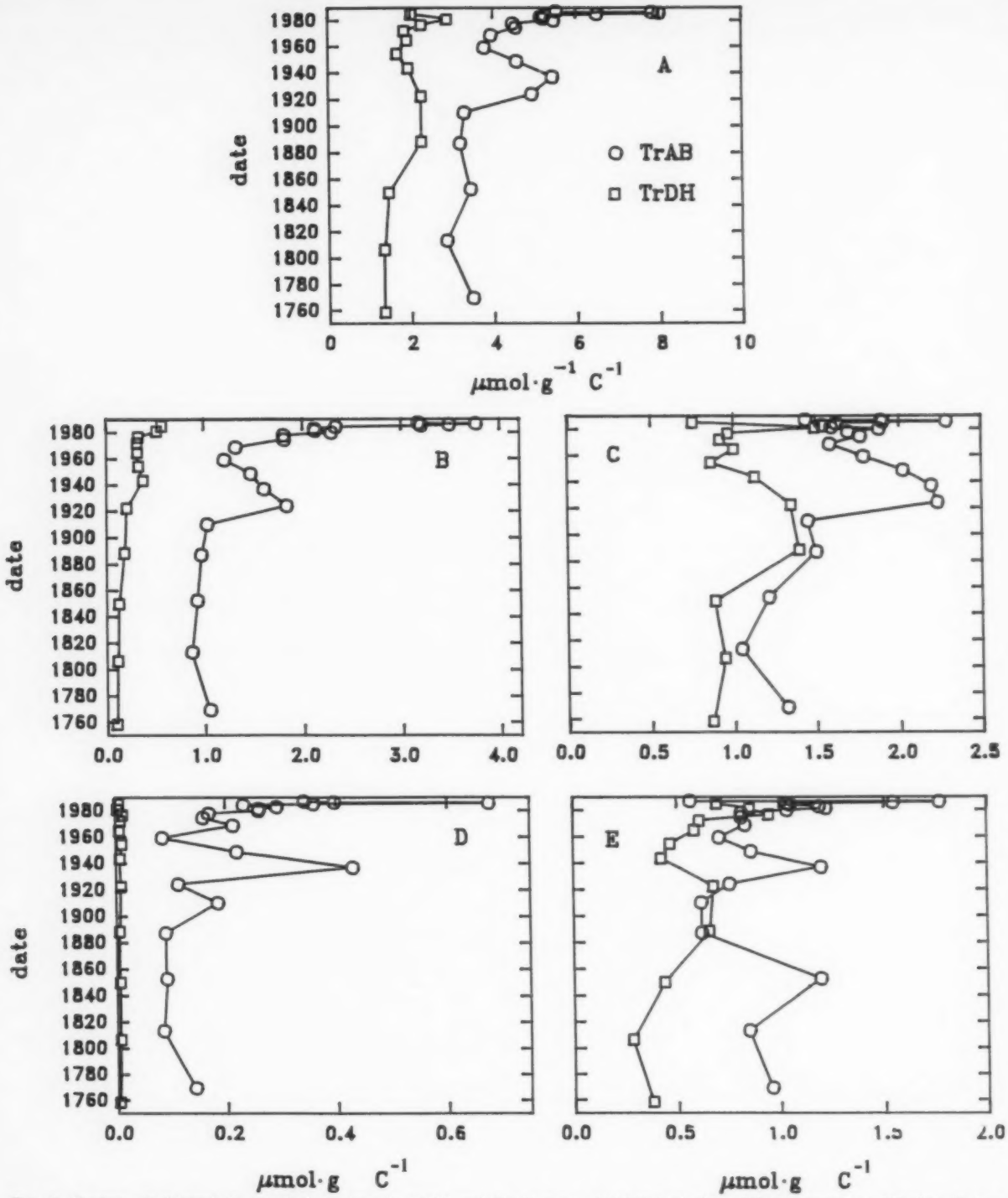

FIG. 5. Content of chlorophyll *a* and derivatives in TrDH and TrAB cores. (A) Total phorbin; (B) chlorophyll *a*; (C) pheophytin *a*; (D) chlorophyllide *a*; (E) pheophorbide *a*.

served in surface sediments. In fact, sediment-calculated accumulation was greater than trap-calculated deposition for these carotenoids in TrAB, resulting in negative values for calculated percent degradation.

Several sources of error are encountered in calculating degradation by comparing sediment trap with bottom sediment fluxes, including incomplete trapping (late fall and winter fluxes not included), variable collection efficiency, focusing correc-

tion errors, and deep production not collected in sediment traps. The latter process has been observed in other small lakes in the area (Hurley and Watras 1991). Use of minimum and maximum focusing correction factors should eliminate focusing errors. Thus, deep production and/or incomplete trapping probably account for apparent "negative degradation" of several carotenoids. Clearly, substantial differences in preservation occur between basins and among individual carotenoids, but estimates of percentages degraded may be biased by uncertainties in flux estimates.

The observation of increased concentrations of several pigments in surface sediments over sediment trap material (Table 4) seems inconsistent with calculated loss through diagenesis. However, loss of particulate material mass during incorporation of material deposited from the water column into surface sediments "concentrates" constituents preserved in sediment. Major losses in mass occur through organic matter decomposition and dissolution of biogenic silica. Calculations based on flux comparisons indicate that losses of deposited mass were about 38–62% in TrAB and 48–65% in TrDH. Total carbon degradation was about 52–70%. Although the sediment accumulation rate exceeded the rate of delivery as measured by sediment traps in TrAB, sediment focusing accounted for 57–84% of the measured accumulation rate. Focusing also influenced TrDH, but the measured accumulation rate was considerably less than the deposition rate, even without correction for focusing. Assuming that calculated focusing-corrected fluxes are accurate, loss of particulate matter mass would increase the concentration of a conservative substance by a factor of 1.6–2.6 in TrAB and 1.5–2.1 in TrDH. Without focusing correction, the factor would be almost 2 in TrDH. Interestingly, the proportions of organic matter and biogenic silica lost through diagenesis in surficial sediments are apparently similar, as the organic carbon and biogenic silica concentrations are similar in sediment trap material and surface sediments (Hurley 1984).

Calculations of diagenetic loss by flux comparisons are dependent on accuracy of flux estimates. Importantly, other evidence also indicates that diagenetic losses have occurred. This evidence is based on comparisons of pigment concentrations, molar ratios, and concentrations relative to organic carbon. Lower concentration in surface sediment than in sediment trap material is a clear indication of pigment loss. For concentration to decrease, percentage loss of pigment must exceed loss of bulk material. As shown by ratios of concentrations in surface sediments to sediment trap material, normalized to organic carbon (Table 4), this is the case for pheophorbide, chlorophyllide, diadinoxanthin, and peridinin in both basins and also for chlorophyll *a*, fucoxanthin, and diatoxanthin in TrDH. Except for pheophorbide, the decrease in concentration is greater in TrDH. Clearly, these pigments have been partially or completely removed by diagenesis and the losses are equal or greater in the oxic TrDH basin. Further, since sediment flux calculations indicate that loss of particulate matter mass by diagenesis is more than 40% in TrAB and 50% in TrDH, we conclude that degradation has occurred where the concentration ratio is less than 1.6 in TrAB and 2.0 in TrDH. This is the case for all pigments except pheophytin (although the sum of chlorophyll and pheophytin shows degradation in TrDH), alloxanthin, lutein + zeaxanthin, and β-carotene, and for diatoxanthin in TrAB. The pattern is similar if mass concentrations rather than organic carbon normalized values are used, since the carbon contents are similar between basins. Ratios would be increased slightly because organic carbon in surface sediments is 75–85% of the sediment trap material concentration.

Pigment Accumulation In Sediments

Mass accumulation rates (Table 5) and pigment concentrations were used to construct profiles of pigment concentrations versus time. Profiles are used to assess relations of surficial diagenesis to downcore pigment concentrations, contrasts between basins, and possible changes due to nondiagenetic factors.

Phorbin accumulation

Total phorbin content, determined by HPLC in this study, is analogous to SCDP units determined spectrophotometrically (Sanger and Crowl 1979). In some cases, SCDP is expressed in units per gram of organic matter to negate dilutional effects of changing levels of inorganic components. Similarly, we have normalized concentrations (Fig. 5A) to organic carbon (measured directly). Concentrations can be expressed on an organic matter basis by dividing values by a factor of about 2.

Although strongly influenced by diagenesis at the sediment surface, phorbins are found deeper in sediment cores. Furthermore, trends in sediments buried longer than 10–20 yr are not suggestive of major diagenetic changes. Total phorbin content (Fig. 5A) in TrAB decreased from about 8 to 3 $\mu mol \cdot g\ C^{-1}$ around 1700–50. A strong subsurface peak observed in TrAB between 1910 and 1950 was less evident in TrDH, where total phorbin content varied only between about 1.5 and 2.7 $\mu mol \cdot g\ C^{-1}$.

Contrasts between basins are apparent in phorbins remaining in surface sediments (Fig. 5A–5E). In TrDH, where particles are retained in the mixed layer for a longer period and subjected to aerobic conditions, diagenesis reduces *a* phorbin content to about 50% of that in TrAB. Chlorophyll and chlorophyllide *a* exhibited the greatest differences (Fig. 5A and 5D). In TrAB, chlorophyll decreased from over 3.5 $\mu mol \cdot g\ C^{-1}$ at the surface to 1 $\mu mol \cdot g\ C^{-1}$ before 1910, while chlorophyllide decreased from over 0.6 $\mu mol \cdot g\ C^{-1}$ at the surface to less than 0.15 $\mu mol \cdot g\ C^{-1}$. In contrast, chlorophyll in TrDH ranged from only about 0.1 to 0.6 $\mu mol \cdot g\ C^{-1}$ and chlorophyllide represented a small fraction of the levels in TrAB. Although pheophytin and pheophorbide *a* levels were lower in TrDH than in TrAB, the differences were much smaller than for chlorophyll and chlorophyllide *a*. Pheophytin concentrations reflect postdepositional formation from chlorophyll *a*, especially in TrDH. Similarly, demetallation of chlorophyllide (Fig. 5D) may account for some of the pheophorbide accumulated (Fig. 5E).

Carotenoid accumulation

Similar to total phorbins (Fig. 5A), total carotenoid content (Fig. 6A) reflects greater preservation in TrAB. Surface sediment concentrations were substantially lower in TrDH (3 $\mu mol \cdot g\ C^{-1}$) than in TrAB (5 $\mu mol \cdot g\ C^{-1}$). The carotenoid profile in TrAB exhibits a subsurface peak at 1910–50, with some carotenoid levels exceeding those of the sediment surface. This carotenoid peak is concurrent with a rise in chlorophyll content (Fig. 5A). Downcore carotenoid levels remain high in TrAB (about 4 $\mu mol \cdot g\ C^{-1}$) compared with TrDH ($<1\ \mu mol \cdot g\ C^{-1}$).

Substantial diagenesis of fucoxanthin is clearly observed in both basins (Fig. 6B). Profiles show a sharp decline in fucoxanthin concentration in the upper section of the core. Fucoxanthin, together with diadinoxanthin and peridinin, contrib-

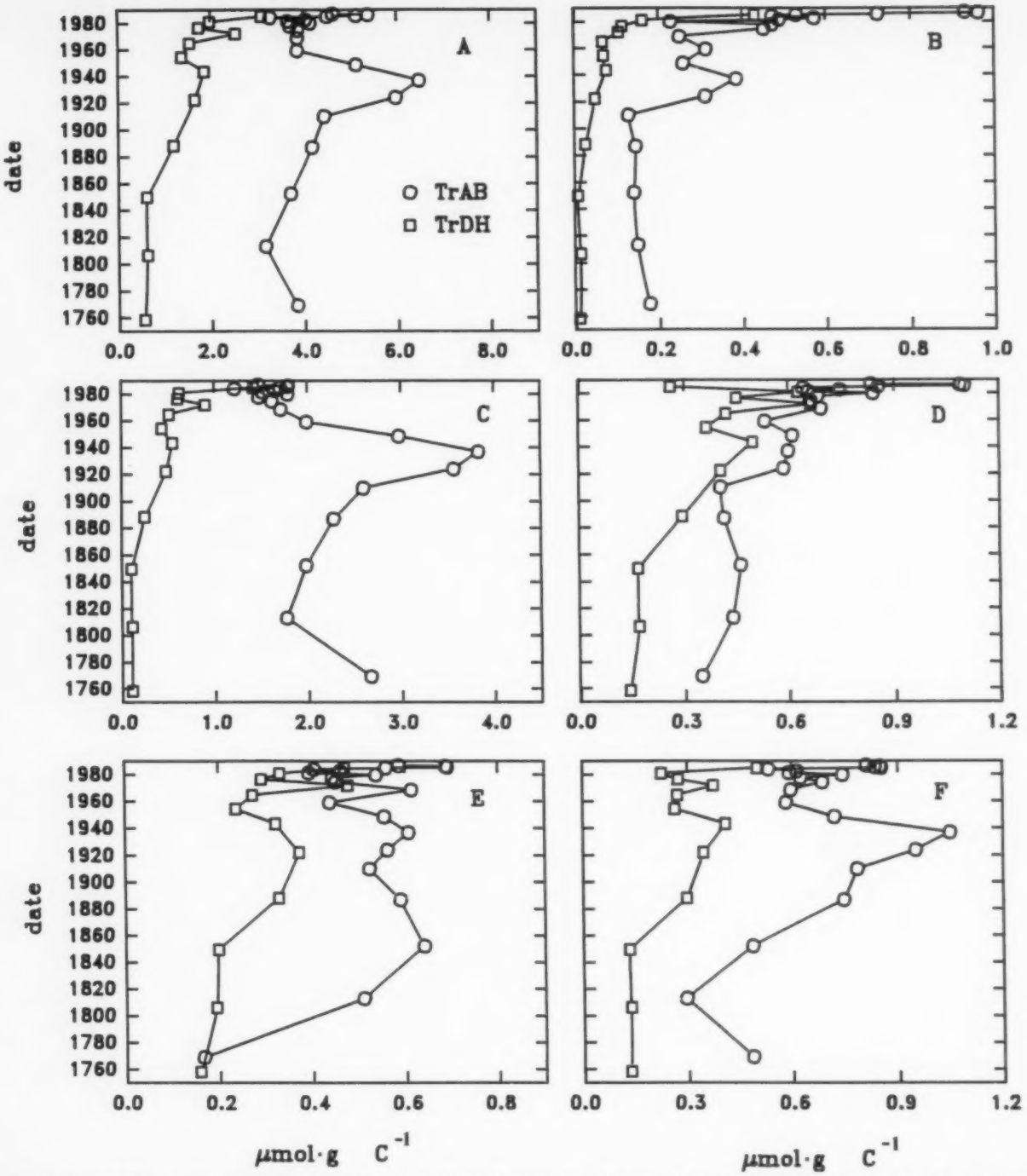

FIG. 6. Content of carotenoids in TrAB and TrDH cores. (A) Total carotenoid; (B) fucoxanthin; (C) diatoxanthin; (D) alloxanthin; (E) Lutein + zeaxanthin; (F) β-carotene.

uted 67–72 mol% of the carotenoid flux to the sediment surface, yet neither diadinoxanthin or peridinin has been preserved in the sediment record. Fucoxanthin levels rise dramatically in TrAB after 1910. A corresponding peak is not evident in TrDH, but a gradual increase occurs after 1890.

Diatoxanthin, a minor carotenoid in diatoms and also a constituent of other algal taxa, appears to be well preserved in TrAB (Fig. 6C). Despite representing only 8–15 mol% of the total carotenoid flux to the sediment surface, diatoxanthin accounted for 25–60% of carotenoids in sediments. A major

subsurface peak centered around 1930 occurred in TrAB. In TrDH, the surface concentration was similar to TrAB (1.5 μmol·g C^{-1}), but the subsurface concentrations were considerably lower. Contrasts between basins in lutein + zeaxanthin and β-carotene profiles (Fig. 6E and 6F) were similar to those for diatoxanthin.

The alloxanthin profile in TrAB (Fig. 6D) suggests rapid surficial diagenesis even though flux calculations (Table 6) indicated nearly complete preservation. Surprisingly, profiles below the SML indicate only moderately higher preservation in the anoxic basin.

It is important to note that profiles of individual pigments plotted versus time have not been corrected for the effects of mixing. Based on the SML depth measured from ^{210}Pb profiles, our estimates of date corresponding to a specific depth or peak may be early by about 32 yr in TrDH and 5.4 yr in TrAB (Table 5) based solely on downward mixing of material deposited into the SML. However, we detect profiles within the SML, as expected when pigment diagenesis is more rapid than ^{210}Pb decay ($t_{1/2}$ = 22 yr). Thus, age corrections for the effects of mixing were not made.

Discussion

The types and amounts of algal pigments incorporated into the permanent sediment record of Trout Lake have been greatly altered by early diagenesis. Fucoxanthin and untransformed chlorophyll *a* were the major carotenoid and *a* phorbin pigments deposited from the water column, indicating that direct settling of Chrysophyta is the major pigment deposition vector in the lake. However, both chlorophyll *a* and fucoxanthin are substantially degraded at the sediment surface (especially in TrDH), confounding direct interpretation of their depositional history. Even so, subsurface peaks of some pigments were evident in TrAB and, to a lesser extent, in TrDH, indicating that some records of changes in pigment deposition have been preserved.

Although important in both basins, diagenesis is more extensive in TrDH than in TrAB and is greatly retarded after pigments are buried below the mixed layer. In TrDH, where particles are retained longer in the mixed layer and subjected to aerobic conditions, diagenesis reduces *a* phorbin content to about one half and total carotenoids to about one fourth that in TrAB (Fig. 5 and 6). However, profile shapes are similar, except for pronounced maxima around 1930. Clearly, anoxia retards degradation at the surface. Partial preservation of pigments in both basins after burial may reflect anoxia and/or low microbial activity (Sanger 1988).

More rapid diagenesis in the surface sediments of TrDH may result from processes favored in an oxygenated hypolimnion, such as ingestion by detritivores, and a longer residence time of particles in the SML of TrDH (Table 5). Detritivore processing and physical mixing can affect pigment degradation in two ways. First, physical transport of subsurface sediments to the sediment–water interface, perhaps through a biological conveyer belt, reexposes the particles to oxygen (Berner 1980). Both demetallation (phorbins) and degradation of phorbins and carotenoids may result. Second, processing by detritivores could produce pheophorbide or carotenoid transformation products (Repeta and Gagosian 1984, 1987), analogous to processes occurring during grazing by zooplankton (Shuman and Lorenzen 1975).

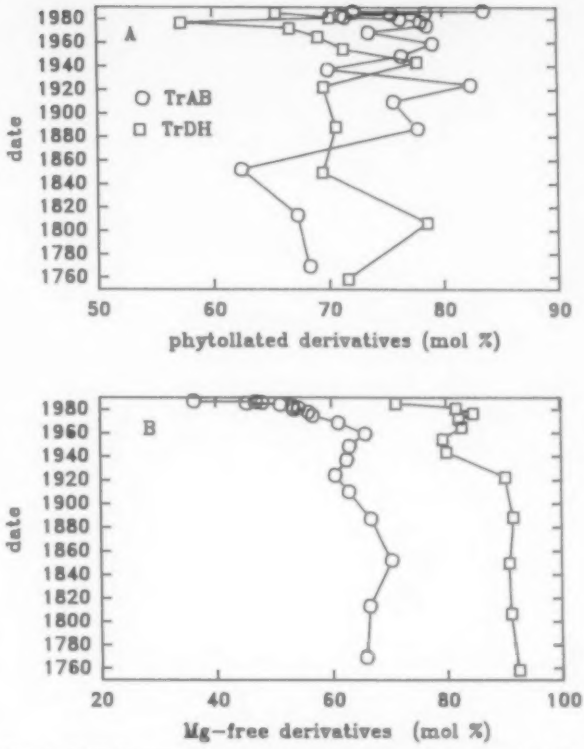

FIG. 7. Phorbin composition (mol%) versus time in TrAB and TrDH cores. (A) Chlorophyll + pheophytin; (B) pheophytin + pheophorbide.

In addition to sedimentary environment, pigment structure also influences phorbin diagenesis. Pheophytin appears to be preferentially preserved. The major conversion of chlorophyll *a* (not including pigment destruction) at the sediment surface in both basins was to pheophytin *a*. The extent of conversion was greater in TrDH. Material delivered to traps in both basins contained between 8 and 17 mol% pheophytin (Table 2), yet in deeper sediments, pheophytin represented about 60 mol% in TrDH and 40 mol% in TrAB (Fig. 5). Conversion to pheophytin concurs with Keely and Brereton's (1986) observation of a steady decrease in the ratio of chlorophyll to pheophytin in the sediments of a eutrophic lake with an anoxic hypolimnion. Preservation of pheophytin may reflect lower susceptibility to degradation and/or postdepositional formation and exposure to conditions favoring rapid diagenesis for a shorter time than for chlorophyll.

The sinking of fecal pellets (containing transformed pheophorbide from chlorophyll) can be an efficient vector for the physical deposition of organic matter and provide an important grazing biomarker (pheophorbide) to the surface sediments. In some cases, pheophorbide is the dominant phorbin delivered to the sediment surface (Welschmeyer and Lorenzen 1985; Carpenter et al. 1986, 1988; Furlong and Carpenter 1988). In Trout Lake, however, this is not the case, as about 50–60 mol% of the phorbin flux to the sediments arrives as untransformed chlorophyll *a* (Table 2), with an additional 15% as pheophytin and 10% as chlorophyllide.

To interpret its significance as a grazing indicator, relative rates of diagenetic pathways influencing sedimentary pheo-

phorbide must be considered. Similar to formation of pheophytin from chlorophyll, demetallation of chlorophyllide (Fig. 5D) may account for some of the pheophorbide accumulated (Fig. 5E), especially in TrDH. The sum of phytol-containing phorbins (chlorophyll + pheophytin) on a molar percent basis (Fig. 7A) is nearly constant between the two basins. The remaining 20–30% of the phorbin fraction is present as chlorophyllide *a* and pheophorbide *a*. However, the proportion of magnesium-free derivatives differs (Fig. 7B), suggesting that demetallation (more extensive in TrDH) is the main factor accounting for differences between basins in proportions of residual sedimentary phorbins. The possibility of diagenetic demetallation complicates interpretation of the significance of pheophorbide *a* in the TrDH core. Postdepositional demetallation of chlorophyllide *a* may have been an important source of pheophorbide. Chlorophyllide *a* is more abundant at the top of the TrAB core (Fig. 5D) and decreases with depth. Absence of significant chlorophyllide *a* in surface sediments of TrDH suggests either rapid degradation or conversion to pheophorbide.

Importantly, loss of detectable phorbins from sediments may not reflect total mineralization. Furlong and Carpenter (1988) noted that opening of the tetrapyrrole macrocycle or incorporation into other organic materials such as humic substances may remove phorbins from the analytical window. Further investigation using different extraction and analytical techniques would be needed to determine whether conversion to nonextractable forms or other degradation products partly accounts for differences in phorbins between the two basins of Trout Lake.

Substantial differences in preservation also exist among the carotenoids, as found in other systems (Repeta and Gagosian 1987; Hurley and Armstrong 1990; Leavitt and Carpenter 1990a, 1990b). Fucoxanthin, the predominant carotenoid delivered to the sediment surface, is substantially degraded, especially in TrDH, diminishing its utility as an indicator of Chrysophyte abundance. In TrAB (Fig. 6B), fucoxanthin increased about 1910, but the subsurface maximum was about 50% less than surface sediment concentrations. However, diatoxanthin (Fig. 6C), a minor pigment in Chrysophytes (as well as Euglenophytes and others), appears to be well preserved, exhibits a major subsurface peak, and may be a better indicator of past Chrysophyte abundance. Retention of alloxanthin, a common carotenoid of cryptomonads, is also high.

The preservation of individual carotenoids in sediments has been linked to pigment structure. Our results concur with the general reactivity scheme postulated by Repeta and Gagosian (1987) that carotenoids containing 5,6-epoxides (fucoxanthin, diadinoxanthin, and peridinin) are degraded more rapidly than those without epoxide substitution (alloxanthin, diatoxanthin, lutein, zeaxanthin, and β-carotene). Both peridinin and diadinoxanthin are completely degraded prior to accumulation in sediments. Residual fucoxanthin in TrAB sediments suggests better preservation of fucoxanthin than of peridinin and diadinoxanthin, yet substantial degradation of fucoxanthin continues downcore (Fig. 6B). Similar losses of 5,6-epoxides were observed in Lake Mendota (Hurley and Armstrong 1990) where peridinin, the most abundant water column carotenoid, was not found in surface sediments. Fucoxanthin, however, was partially preserved. We conclude that higher fucoxanthin levels at the tops of both Trout Lake cores (Fig. 6B) reflect diagenetic removal during burial rather than recent increased input. The increase between 1910 and 1950 (TrAB) may reflect a period of higher fucoxanthin input, since the peak occurs well below the SML. The subsurface peak suggests that fucoxanthin may be a viable paleomarker in seasonally anoxic sediments with high sedimentation rates relative to particle retention times in the SML. The differences in P_{deg} of 51–70% in TrAB, as compared with 92–95% in TrDH (Table 6), are reflected in downcore retention. However, even in TrAB, the profile shows a greater decrease (four- to fivefold) in concentration below the sediment surface than for other preserved carotenoids (Fig. 6C–6E).

In contrast with epoxide-containing carotenoids, diatoxanthin, alloxanthin, lutein, zeaxanthin (all containing 3,3′-hydroxyl groups on end rings), and β-carotene appear to be less susceptible to degradation (Table 5) and offer better possibilities for paleolimnological reconstruction. Diatoxanthin (Fig. 6C) is apparently well preserved in TrAB. Surface concentrations are similar to those in deeper portions of the core, and a large subsurface peak is clearly evident. Sharp decreases from surface sediment levels are observed in TrDH, similar in magnitude to fucoxanthin, suggesting susceptibility to degradation in this sedimentary environment. Alloxanthin shows similar trends between basins. In contrast with Repeta and Gagosian (1987), diatoxanthin and alloxanthin may not be useful in TrDH for diatom/cryptomonad assemblage reconstruction due to differences in preservation. In TrAB, however, the two carotenoids appear to be preserved to a similar degree and direct comparison may be appropriate.

Three carotenoids (lutein, zeaxanthin, and β-carotene) appeared to be relatively well preserved. However, these pigments do not clearly differentiate specific taxa of algae. Lutein, present in green algae, coelutes with zeaxanthin, a potential blue-green marker. β-Carotene is nonspecific, being present in all classes of algae. Structure likely accounts for preservation of these carotenoids. Both lutein and zeaxanthin are structurally similar to diatoxanthin (3,3′-dihydroxy-), while β-carotene has no oxygen-containing functional groups.

Although baseline data collected in Trout Lake from 1980 to present (LTER 1989) provide a potential opportunity for assessing relationships between water column processes and sedimentary pigments, sediments deposited during this period are contained within the SML where it is difficult to resolve the effects of mixing, diagenesis, and depositional changes. Over longer time scales (last 100 yr), however, comparisons may be informative. In particular, the subsurface peak centered around 1930 for total phorbins (Fig. 5A) and total carotenoids (Fig. 6A) likely reflects a change in pigment production and deposition, rather than a diagenetic event. The peak is prominent in TrAB and also evident in TrDH.

Located within the Northern Highlands and American Legion State Forest, development around the Trout Lake drainage basin is not extensive. Neither sewage treatment facilities nor agricultural practices, common causes of cultural eutrophication, were developed within the basin. Nutrient levels observed recently (Hurley 1984; LTER 1987) are similar to those found in the early 1930's (Juday and Birge 1931; Juday et al. 1938). However, between about 1910 and 1920, most of the drainage basin was clear-cut for lumber production and burned (Curtis 1959; Webb 1973). At the same time, erosion, nutrient loading, and lake productivity probably increased. Other work has shown increased loadings of phosphorus (Wood et al. 1984) and nitrogen (Krause 1982; Swank 1987) in streams following clear-cutting of surrounding forests. Apparently, clear-cutting in the Trout Lake region increased lake productivity and sedi-

mentary deposition of total phorbins (Fig. 5A) and total carotenoids (Fig. 6A). Furthermore, residual diatoxanthin (Fig. 6C) and, to a lesser extent, fucoxanthin (Fig. 6B) profiles in TrAB indicate that chrysophytes were dominant in the phytoplankton deposited.

Although subsurface peaks of fucoxanthin and diatoxanthin suggest shifts in phytoplankton, sedimentary carotenoid degradation products (Repeta 1989; Repeta and Gagosian 1984, 1987) might reflect additional details and community changes. Repeta (1989) has postulated that loliolides found in Peruvian sediments are formed by anoxic degradation of xanthophylls in the presence of sulfate reduction, while saturation of carotenoids to form carotane or partial saturation may occur if methanogenesis is the dominant metabolic process. These transformation products could aid paleolimnological reconstruction of phytoplankton that produce labile precursor carotenoids.

Diagenetic processes produce major, pigment-specific alterations in the deposited pigment flux during incorporation into the permanent sediment record of Trout Lake. Diagenetic rates are accelerated in bioturbated, oxic sediments of TrDH, relative to seasonally anoxic sediments in TrAB. Additionally, increased sedimentation rates resulting from sediment focusing in TrAB produce shorter periods of retention in the SML and major differences in pigment profiles, both in distribution and concentration. Use of basin- and pigment-specific data on rates of degradation in surface sediments with diagenetic models (Berner 1980; Emerson 1985) should improve quantitative assessment of sedimentary pigments for paleolimnology.

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A New Method of Estimating Gillnet Selectivity, with an Example for Spotted Seatrout, *Cynocion nebulosus*

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Helser, T. E., R. E. Condrey, and J. P. Geaghan. 1991. A new method of estimating gillnet selectivity, with an example for spotted seatrout, *Cynocion nebulosus*. Can. J. Fish. Aquat. Sci. 48: 487–492.

This study proposes a new method of estimating the selectivity of gill nets. Using data on the distribution of fish lengths from the catches of various meshes in an experimental gill net, a system of m equations and n unknown coefficients is simultaneously solved describing the functional relationship of the moments from an assumed mathematical model across mesh size and size-class of fish. The solution to the model is a response surface describing the probability of capture of fish of size-class j in mesh i. This new method is applied to spotted seatrout (*Cynocion nebulosus*) in coastal Louisiana and used to estimate the relative abundance of the various size-classes of fish in the population. We suggest that this approach for estimating gillnet selectivity has several advantages over other methods: it is very flexible in terms of choosing a mathematical model which would best represent the data; it takes full advantage of the empirical data allowing catch information, n_{ij}, to contribute to the final solution both as a function of mesh size and size-class; and it offers simplicity and elegance in its overall iterative least squares approach and is tractable in terms of assessing the reliability of the parameters and model used.

La présente étude porte sur une nouvelle méthode d'estimation de la sélectivité des filets maillants. D'après des données sur la distribution des longueurs des poissons capturés dans divers maillages d'un filet expérimental, on a résolu simultanément un système de m équations et de n coefficients inconnus décrivant la relation fonctionnelle des moments issus de modèles mathématiques hypothétiques mettant en relation le maillage et la classe de taille des poissons. La solution du modèle est une surface de réponse décrivant la probabilité de capture d'un poisson appartenant à la classe de taille j dans un maillage i. Cette nouvelle méthode est appliquée à l'acoupa pintade (*Cynocion nebulosus*) des eaux côtières de la Louisiane afin de déterminer l'abondance relative des diverses classes de taille dans la population. Selon les auteurs, cette méthode d'estimation de la sélectivité de filets maillants possède plusieurs avantages par rapport aux autres méthodes: elle est très flexible en termes de choix d'un modèle mathématique qui exprime mieux les données; elle profite entièrement des données empiriques car l'information n_{ij} sur les prises contribue à la solution finale comme fonction du maillage et de la classe de taille; son utilisation de la méthode des moindres carrés est simple et élégante; et elle est souple pour ce qui est de l'évaluation de la fiabilité des paramètres et du modèle utilisés.

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Experimental gill nets are often employed as fishery-independent techniques for sampling fish populations and monitoring fish stocks. Experimental gill nets circumvent size selectivity biases encountered when using gill nets of a single mesh size by fishing a series of different meshes (varying only slightly in size) simultaneously so that a broader range of size-classes in the population appear in the catch and the various size-classes of fish are represented in each of the meshes. Selectivity is the usual information obtained from gillnet studies which can be used to estimate the relative abundance of the various size-classes of fish in the population (Olsen 1959; McCombie and Fry 1960; Gulland and Harding 1961) and is needed in managing a commercial gillnet fishery (McCombie 1961; Jensen 1972; Ehrhardt and Die 1988).

Lagler (1968) defined the size selectivity of a gear to be the proportion of the total population of a certain size-class of fish which is caught and retained by a unit operation of fishing. Based on this definition, selectivity is the quantitative description of selection by a gear and is usually represented as the probability of capture of a certain size of fish in a certain-sized mesh. If the true population size structure cannot be directly determined or accurately estimated, then one must resort to indirect methods of estimating the selectivity of the meshes. Most of the indirect methods which have been proposed in the literature follow one of two basic approaches: the use of "type A" curves with methods developed by Olsen (1959), Holt (1963), and Hamley and Regier (1973) or methods which utilize "type B" curves as intermediaries such as that of Baronov (1948), McCombie and Fry (1960), and Regier and Robson (1966). Recently, another indirect iterative method has been proposed by Boy and Crivelli (1988) which requires an estimate of the population age structure. Regier and Robson (1966) defined type A selectivity to represent the probability of capture of one mesh size to various size-classes of fish, while type B selectivity gives the probability of capture of a single size-class of fish to different meshes. With suitable assumptions, various

mathematical models have been used to estimate the shape of type A selectivity curves, and Regier and Robson (1966) and Hamley (1975) provided a thorough review of these methods. We found the methods which use the type A curve approach unsuitable for our data on spotted seatrout (*Cynocion nebulosus*) because of departures from the assumption of normality and/or incongruity between curves. Among those methods which use type B curves, Regier and Robson (1966) found their computational approach better at estimating the selectivity of a 4.5-in. (11.4-cm) mesh for lake trout based on a known standard.

The model presented here arose out of an attempt to solve Regier and Robson's (1966) model by applying a nonlinear iterative least squares approach to estimating the selectivities both as a function of mesh size and size-class of fish. The primary reasons for pursuing this different approach are twofold. First, it was felt that an iterative least squares fitting procedure to the actual catches of fish of size j in mesh i might better describe the systematic change in type A and type B catch distributions simultaneously, i.e. fitting an overall response surface. Second, the approach would be tractable in terms of assessing the model's fit to the empirical data as well as the reliability of the parameter estimates.

We applied this new nonlinear iterative least squares fitting procedure to experimental gillnet catches of spotted seatrout and estimated the selectivity of the different meshes fished. The selectivities derived from the model are used to estimate the relative abundances of the various size-classes of fish in the seatrout population and the gillnet selection characteristics which are needed parameters to assess the status of the spotted seatrout stocks in coastal Louisiana.

Materials and Methods

This study is based on experimental gillnet data obtained from a coastal finfish monitoring program conducted by the Louisiana Department of Wildlife and Fisheries. State biologists employed a run-around method to capture spotted seatrout in monofilament nylon experimental gill nets in one of five possible mesh sizes: 2.0, 2.5, 3.0, 3.5 and 4.0 in. (5.08, 6.35, 7.62, 8.89, and 10.16 cm, respectively) stretch measure. Fish tangled in the nets (768 fish), identified as those held in the net anterior to the gill covers or on body protrusions, were excluded and the 1988 gillnet catches of spotted seatrout used for analysis. A brief description of fishing and data collection procedures will be provided here; however, readers interested in specific methodology should consult Adkins and Bourgeois (1982).

Data were collected by teams of finfish biologists within seven hydrographic areas covering the entire coastal zone of Louisiana. Approximately 8–14 stations within each zone were sampled twice monthly between January and October 1988. At each station, experimental gill nets, measuring 750 ft (228.6 m) in length (150 ft per mesh panel), were deployed by biologists and encircled approximately three times by the fishing vessels to drive fish into the nets. The nets were then retrieved, starting with the panel first entering the water, and the catch enumerated by mesh. Fish were measured to the nearest millimetre, weighed to the nearest gram, and examined for stomach content and maturity stage.

List of Symbols

l_0 = mean size of fish caught in m_i.

σ_i = standard deviation of the catches of fish size-class j in mesh i.

q_i = skewness coefficient of the catches of fish size-class j in mesh i.

l_j = mean size of fish in size-class j.

m_i = size of mesh i; specifically, stretch measure in inches.

n_{ij} = catches of fish of size-class j in mesh i.

$n_i = \sum_j n_{ij}$

N_j = relative number of fish of size-class j in the population.

p_{ij} = relative frequency of fish of size-class j in mesh i, i.e. $n_{ij} / \sum_i n_{ij}$.

s_{ij} = selectivity of mesh m_i to fish of size-class l_j.

S_{ij} = standard selectivity or probability of capture, i.e. $s_{ij}/\max_i(s_{ij})$.

Skew-normal probability density function:

$$s_{ij} = \frac{1}{\sigma\sqrt{2\pi}} \exp^{-(l_j - l_0)^2/2\sigma^2} \times \left\{1 - 1/2q\cdot\sigma^{3/2}\left[\frac{(l_j - l_0)}{\sigma} - \frac{(l_j - l_0)^3}{3\sigma^3}\right]\right\}.$$

Model Development

Hamley (1975) stated that the shape of the type B selectivity curve can be estimated without bias by comparing the catches for fixed l_j across different m_i. Because the individual meshes themselves were fished with equal effort, we assume that the probability of encounter of fish of a single size-class is equal with respect to the different meshes and that each mesh caught the same proportion of fish of the size-class for which that mesh is most efficient (equal catchabilities at the peak of each curve). Therefore, catches of a single size-class are proportional to the selectivities and s_{ij} can be determined by fitting a likely mathematical model on m_i for fixed l_j. This is refered to as type B selectivity (Regier and Robson 1966).

In addition to the probability of capture, we also use the term probability of occurrence to mean, on the average, the expected catch of fish of size-class j in mesh i given the size/age structure over the period of the study. Thus, the probability of occurrence of the various size-classes of fish is a function of the population size structure and may change from one year to the next.

The probability of l_j occurring in m_i is roughly the proportion of fish caught of that size to n_i. The proportion across all size-classes in a given mesh resembles the size frequency distribution of mesh i and can be modeled using a variety of probability functions. Baronov (1948) envisioned the size distribution of catches from gill nets to look like a normal probability function, as the mesh of a gill net would capture fish of a definite size and few whose length differed from the optimum by more than 20%. However, gillnet size frequency distributions are frequently either positively or negatively skewed and even multimodal.

For this particular data set, we chose a skew-normal probability density function because of the pronounced skew associated with the distribution of catches from the meshes in our experimental gang. Regier and Robson (1966) used the skew-normal model to estimate the shape of type B selectivity curves, which they used as intermediaries to determine type A selectivity. This is essentially a normal probability function corrected for skewness using the first two terms of a Gram–

Charlier series (Croxton et al. 1967). This is a three-parameter function whose shape is determined by the mean (l_0), standard deviation (σ), and skewness (q). We wish to emphasize that the general procedure develped here can be used with other mathematical models, and one should be chosen which best describes the form of the data. Such models might include normal, log-normal, gamma, or the Weibull, particularly if a more flexible form is needed to fit the data.

Once an appropriate mathematical model has been selected, the approach is to solve simultaneously a system of m equations which describe the functional relationship of the moments of the catch distributions across m_i and l_j, using a nonlinear iterative model which seeks a least squares solution between the expected catch based on the model and the observed catch of fish. We propose the following method to solve this system.

Step 1. Calculate the moments of each distribution corresponding to the various mesh sizes fished. From our assumed probability function, we would estimate l_0, σ, and q. (We used PROC UNIVARIATE in SAS, Statistical Analysis System; SAS Institute Inc. 1985).

Step 2. Estimate the relationsip of l_0, σ, and q to m_i using multiple linear regression methods.

Step 3. Using the coefficients of the regression equations obtained in step 2 as initial parameter estimates, iteratively solve the system of m equations and n unknown coefficients to obtain a final set of parameters. The nonlinear iterative phase of the model seeks a least squares solution between the observed catches, n_{ij}, and the predicted catch of fish from the model (right-hand side of the equation). We used PROC NLIN in SAS with the weighting factor, p_{ij}, and the following model:

$$n_{ij} = \frac{n_i}{\sigma\sqrt{2\pi}} \exp^{-(l_j - l_0)^2/2\sigma^2} \times \left\{1 - 1/2q\cdot\sigma^{3/2}\left[\frac{(l_j - l_0)}{\sigma} - \frac{(l_j - l_0)^3}{3\sigma^3}\right]\right\}.$$

Step 4. Obtain an estimate of selectivity, s_{ij}, by inserting the final estimated set of n coefficients and the system of m functional equations from step 3 into the assumed probability function.

Step 5. Standardize the ordinates, s_{ij}, of each curve by multiplying by the factor $d/\max_i(s_{ij})$, where $\max_i(s_{ij})$ is the ordinate at the mode of the curve and d is an arbitrary constant. This adjusts each curve to agree with the assumption that, at peak efficiency, nets of different mesh size are equally efficient.

Results and Discussion

The moments derived from the catch distributions of spotted seatrout varied systematically with increasing mesh sizes used in our experimental gang. The mean exhibited a strong positive linear relationship ($r^2 = 0.99$), while both the standard deviation and skewness varied across mesh as a second-order polynomial (Fig. 1). The coefficient of determination (r^2) was 0.98 and 0.96 for the standard deviation and skewness, respectively. The above functional relationships between the moments and mesh size provided the system of three equations and eight initial parameter estimates used in the nonlinear iterative phase of the model.

The final set of iteratively solved parameters from the nonlinear phase of the model were in general close to the initial parameter values (Table 1). The precision associated with the final parameter solutions in Table 1 indicated good model performance. Additional indications of model performance are that generally no more than 100 iterations were needed to meet convergence and the final set of iteratively solved parameters in the model explained no less than 98% of the total variation in the data. All eight parameters used in the model were significantly different from zero with generally small asymptotic standard errors (Table 1). Five of the eight initial values were within one standard error of the final parameters and all were within asymptotic 95% confidence intervals. The final set of equations represents an improvement over the initial parameter estimation as the weighted sums of squares were reduced by 74%. Relatively small differences between the initial and final

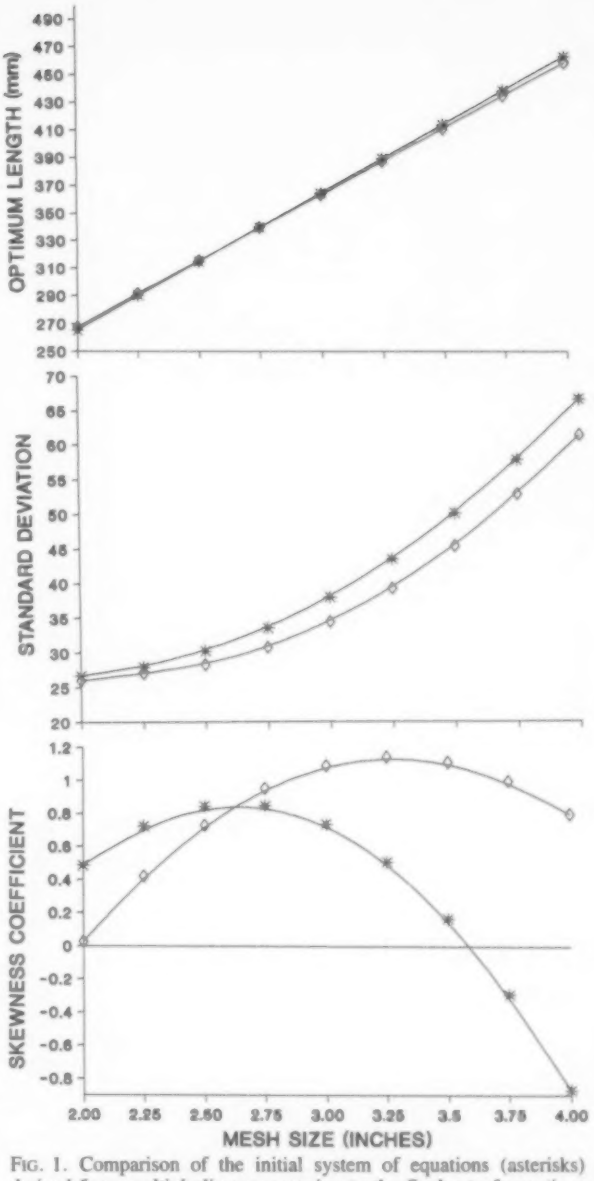

FIG. 1. Comparison of the initial system of equations (asterisks) derived from multiple linear regression to the final set of equations (diamonds) estimated from the nonlinear iterative phase of the model.

TABLE 1. Model performance showing precision of the estimated coefficients for the final system of equations. Initial and final coefficients are compared to assess changes which result from the nonlinear iterative phase of the model.

Parameter	Functional form	Coefficient		Asymptotic 95% confidence	
		Initial	Final	Lower	Upper
Mean	$\alpha + \beta_1 \cdot m_i$	68.86	75.90	66.59	85.21
		99.20	95.81	91.78	99.83
Standard deviation	$\alpha + \beta_1 \cdot m_i + \beta_2 \cdot m_i^2$	56.34	70.81	48.84	92.77
		−32.13	−41.42	−59.95	−22.88
		8.64	9.76	5.87	13.64
Skewness	$\alpha + \beta_1 \cdot m_i + \beta_2 \cdot m_i^2$	−5.61	−6.17	−9.17	−3.17
		4.91	4.45	2.13	6.78
		−0.93	−0.68	−1.12	−0.23
Model $r^2 = 0.98$			74% reduction in type III sum of squares		

equations, shown graphically in Fig. 1, indicate stability in the final parameter solutions. No substantial differences are observed between these equations for the mean, although both initial coefficients (intercept and slope) were between the one and two standard error range of the final solution. The final solution to the equation for the standard deviation appears only slightly different from the initial coefficients, resulting in overall decreased values for all but the 2.0-in. meshes. The most pronounced differences appear to have occurred in the skewness coefficient equation. Here, skewness coefficients corresponding to larger mesh sizes (3.0–4.0 in.) shifted up, while the two smaller meshes shifted down with all values being positive.

The above statistical considerations suggest that the system of three equations and eight parameters used with the skew-normal model was suitable for the spotted seatrout data set used as an example here. This may be in part due to the robustness of the data set (large catches in the meshes fished). Poor precision associated with the parameter estimates and large changes between the initial and final functional equations may indicate instability due to small sample sizes in the meshes. In general, one should chose as simple a function as possible so as not to overparameterize the model, particularly if the data set is not very robust.

From these final equations, the predicted catches derived from the model for each mesh were computed; the expected catches were in general agreement with the empirical data (Fig. 2). The 2.0- and 2.5-in. meshes fit particularly well, while slight departures are observed in the 3.0- and 3.5-in. meshes. Fits are poor with the 4.0-in. mesh, a condition which is probably in part due to the small numbers of fish caught in that mesh relative to the others and the observed bimodal catch distribution.

To the extent that the adjustments from the initial to final equations, using the weighting factor (p_{ij}) and this modeling approach, have filtered out the effects of unequal abundances, the final set of equations inserted into the probability function should produce a fairly reliable representation of the selectivities. We feel that the effects of abundance are to some extent accounted for by the model and that the resultant curves (Fig. 3), with the exception of the 4.0-in. mesh, should provide reasonable estimates of the mesh selection characteristics for spotted seatrout in coastal Louisiana. The selectivity curves shown in Fig. 3 have been standardized (equal heights) under the assumption that the nets fish equally efficient. This assumption may not hold under biases due to net saturation or size-related avoidance by seatrout. We feel that the run-around method employed in this study circumvents net saturation effects, particularly becuse catch rates were less than 25 fish per set for 90% of the sets and rarely exceeded 100 fish per set. Size-related avoidance by seatrout is much harder to assess with the data presented here; however, we would like to address this question in the future. It should be noted from Fig. 3 that these curves are not congruent, an assumption often implied in other indirect methods, and that the selection range increases with increasing mesh size. The relationship between optimum selection length (peak of the curve) and the size of mesh is clearly linear (Fig. 1 and 3). This is a selection characteristic of interest in managing a gillnet fishery, and its theoretical relationship was established by Baranov (1948), i.e. $\phi = KL_m$, where ϕ is the mesh bar measure and L_m is the modal length of fish captured in it and K is called the selection factor. The regression line presented in Fig. 1 is inverse to Baranov's relationship. However, we computed the selection factor for spotted seatrout by regressing ϕ on L_m and found that $K = 0.13$ which was within the range of values found for slim-bodied fishes (0.10) such as mackerel to deep-bodied fishes (0.20) such as bream (Andreev 1962). The theoretical line established here for spotted seatrout can be used to estimate similar optimum selection lengths for mesh sizes not included in this study.

The considerable overlap between selectivity curves from adjacent meshes (Fig. 3) demonstrates that this particular configuration of gear is highly efficient at sampling the seatrout population, as the probability of capture of fish within the mesh series is no less than 70%. The mesh series used here should adequately sample the spotted seatrout population beginning from 250 mm (peak of 2.0-in. mesh) up to about 460 mm (peak of 4.0-in. mesh) which correspond to ages 3 and 4 for male and female fish, respectively (Weiting 1989). Thus, the effectiveness of this experimental gill net to sample older seatrout quickly diminishes beyond those ages; however, the relative population abundance for the various size-classes of fish up to that size can be estimated. More reliable estimates of abundance will be derived from the selectivity curves within the size range where at least two meshes overlap sufficiently (250- to 500-mm size range). Relative abundances were computed here following a convention similar to that of Gulland and Harding's (1961) graphical method and are computed as $N_j = \Sigma(n_{ij}/S_{ij})$.

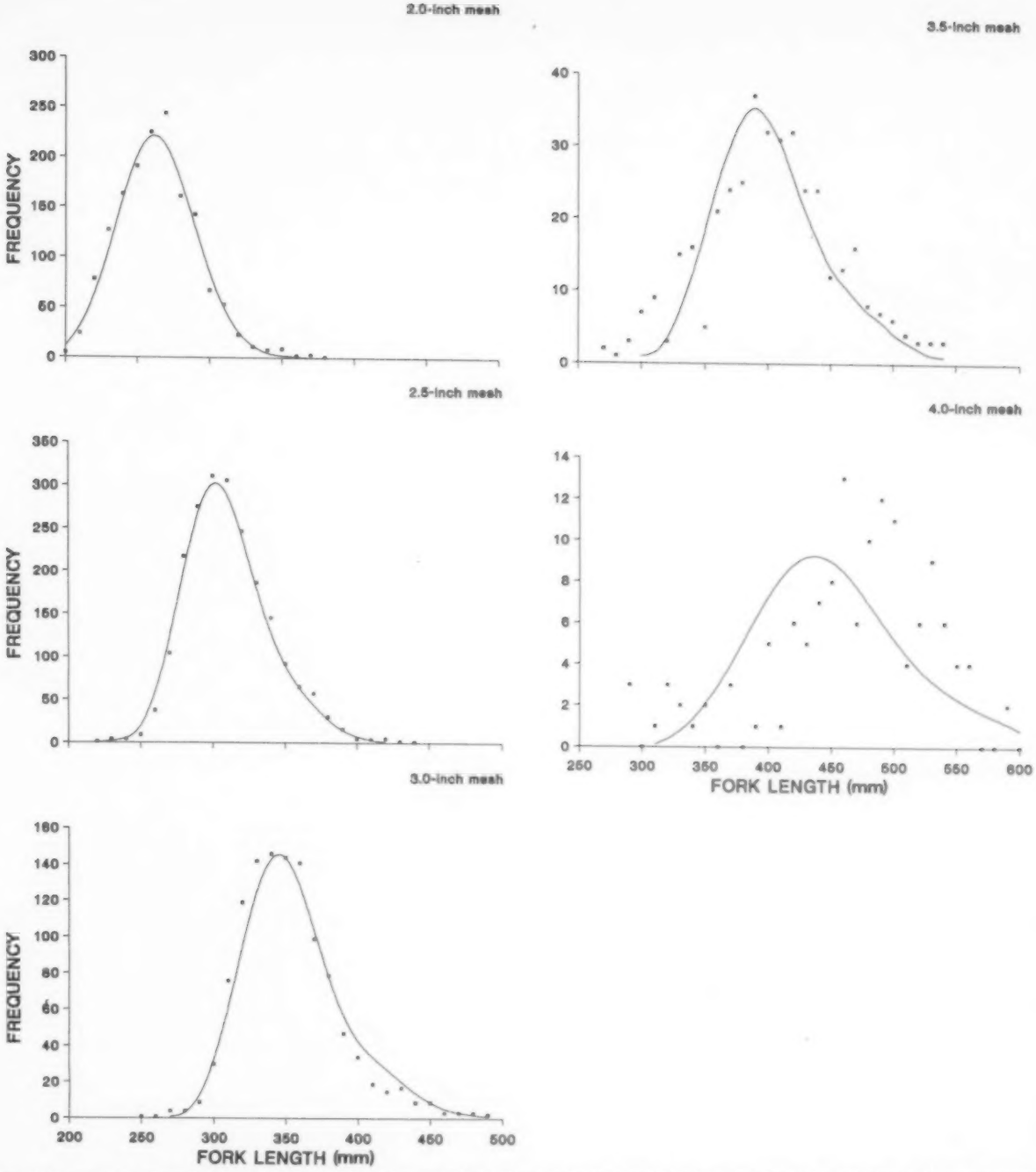

FIG. 2. Comparison of predicted catches derived from the model (solid line) to the observed data (squares) for each mesh size in the experimental gill net.

The estimated relative population size-class abundance is plotted along with the experimental gillnet catches in Fig. 4.

Caution should be used in interpreting the population size structure of spotted seatrout outside the specified range, as small capture probabilities may lead to serious overestimates of abundance. However, inferences concerning the population abundance within the 250- to 500-mm size range are possible and may provide useful information in the assessment of a fish stock. Here it is clear that the observed catches are similar to the true population abundance only over a short size range of fish (350–400 mm). Highest catches observed in the gill nets were in general not coincidental with highest abundances in the population, and declines in abundance appear steeper than would be suggested from the catches.

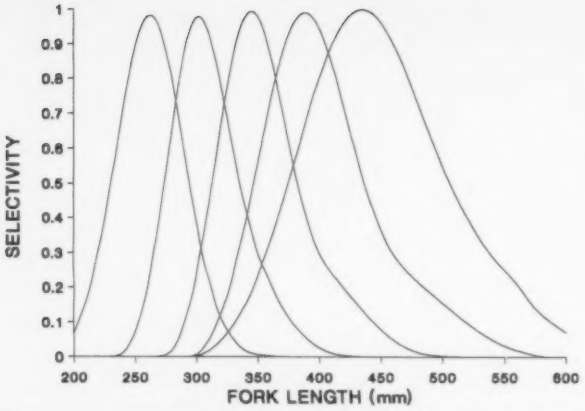

FIG. 3. Estimated selectivity curves of the 2.0-, 2.5-, 3.0-, 3.5-, and 4.0-in. mesh sizes for spotted seatrout. The ordinate, selectivity, is standardized and represents the probability of capture.

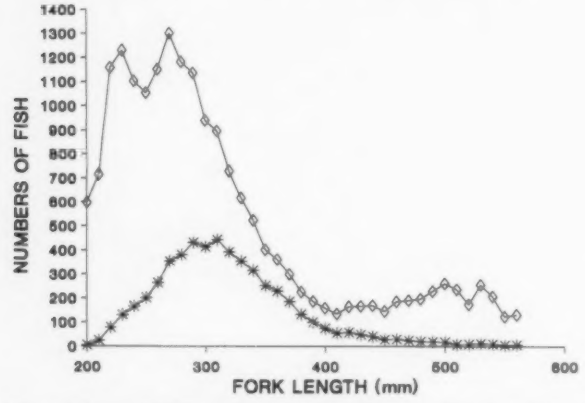

FIG. 4. Observed 1988 experimental gillnet catches of spotted seatrout (asterisks) and the estimated relative abundances (diamonds) of the various size-classes in the seatrout population.

The most striking feature of Fig. 4 is the declining abundances of spotted seatrout from about 300 to 400 mm. These results may have two important management implications for this stock (as suggested from Fig. 4). First, the dramatic declines in population abundance between 300 and 400 mm are coincident with the size at full recruitment to both the recreational and commercial fisheries for spotted seatrout in Louisiana. Second, the most significant spawning contribution to stock replenishment is from females whose length is greater than 400 mm (Arnoldi 1984; Weiting 1989). If the removal of fish from the spotted seatrout population is indeed due to the combined exploitation by the recreational and commercial fisheries, then resource managers may wish to consider management measures to ameliorate fishing mortality within this size range where female seatrout have not yet reached their full spawning potential.

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Effect of Temperature, Season, and Fish Size on Acute Lethality of Suspended Sediments to Coho Salmon (*Oncorhynchus kisutch*)

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Servizi, J. A., and D. W. Martens. 1991. Effect of temperature, season, and fish size on acute lethality of suspended sediments to coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 48: 493–497.

Tolerance of underyearling coho salmon (*Oncorhynchus kisutch*) to Fraser River suspended sediments (SS) at 7°C was independent of season of the year. However, coho of 0.52 g (4.0 cm) possessed only 35% of the tolerance of larger specimens. Tolerance to SS was temperature dependent, with 96-h LC50 at 1 and 18°C being 47 and 33%, respectively, of the value at 7°C. Tolerance was further reduced among underyearling coho which were later found to have a viral kidney infection. Cough reflex, oxygen transfer, oxygen saturation levels, metabolic rates, and capacity to do work all probably affect the relationship between SS tolerance and temperature.

La tolérance des tacons 0+ du saumon coho (*Oncorhynchus kisutch*) aux sédiments en suspension dans les eaux à 0,7°C du fleuve Fraser est indépendante de la saison. Toutefois, les niveaux de tolérance des cohos de 0,52 g (4,0 cm) ne dépassent pas 35 % du niveau noté chez les plus gros individus. Le niveau de tolérance aux sédiments en suspension dépend de la température; ici, la CL50 (96 h) à 1 et 18°C s'élève à 47 et 33 %, respectivement, de la valeur observée à 7°C. Le niveau de tolérance était davantage réduit chez les tacons 0+ qui se sont révélés atteints d'une infection virale du rein. Le réflexe de la toux, le transfert d'oxygène, les niveaux de saturation en oxygène, les rythmes métaboliques et la capacité de travail influent probablement sur la relation entre le niveau de tolérance aux sédiments en suspension et la température.

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Anthropogenic sources and natural events separately and in combination cause elevated levels of suspended sediments (SS) in streams inhabited by Pacific salmon (*Oncorhynchus* spp.). Peak values in excess of 10 $g \cdot L^{-1}$ were reported for a survey of streams in the northwestern United States (Flaxman 1959). Owing to their life cycle, juvenile coho salmon (*O. kisutch*) may be exposed to high SS concentrations at temperatures which range from winter lows to summer highs.

Lethalities of various types of SS to Pacific salmon have been measured. Servizi and Martens (1987) reported that lethalities of Fraser River sediments varied with particle size. Newcomb and Flagg (1983) measured the lethality of volcanic ash. In each of these studies, testing was confined to a narrow range of temperatures. Noggle (1978) conducted tests in the range 6–15°C and suggested that temperatures may influence the tolerance of juvenile coho to SS. However, owing to highly variable SS during testing, the data were inconclusive.

The objective of this study was to define lethal and near-lethal values for SS over a natural temperature range experienced by juvenile coho.

Methods

Test sediment was taken from the same stockpile of lower Fraser River fine sediments described by Servizi and Martens (1987). Particle-size distribution was measured at the Water Resources Branch sediment survey laboratory in New Westminster, B.C., Canada, using the bottom withdrawal tube (BWT) method (Anonymous 1988).

Bioassays (96 h) were conducted in 35-L inverted cone-shaped vessels (42 cm × 52 cm high) partially submerged in a temperature control bath and fitted with recirculation pumps to maintain sediments in suspension. Test fish were confined to cylindrical cages (30 cm × 8 cm high) placed 18 cm below the surface. Fish were confined at a consistent depth to minimize variability of SS exposure concentration and to assure that exposures and SS measurements were as close to each other as practical, as explained below. Samples for SS were collected twice daily at the top of the cages, or when cages were absent, at the surface and 8 cm using a 23-mL cylinder (2.7 cm × 4.5 cm high), which was opened and restoppered underwater. In one case, at 18°C, the fish were not confined to cages but were restricted to a zone from the surface to 26 cm. Contents of the cylinder were vacuum filtered through tared glass fibre Whatman GF/C filters, dried to 105°C for 1 h, cooled in a desiccator, and weighed to the nearest 0.01 mg.

Temperatures in test vessels were measured daily. Dissolved oxygen exceeded 90% of saturation in all cases. Water from Cultus Lake was used for rearing fish and for dilution water. This water has been tested regularly for 25 yr and is stable with respect to pH (7.8), conductivity (168 $\mu S \cdot cm^{-1}$), alkalinity (60 mg $CaCO_3 \cdot L^{-1}$), hardness (84 mg $CaCO_3 \cdot L^{-1}$), and turbidity (2 NTU). The pH was unaffected by the SS concentrations tested.

Coho eggs were obtained from the Chilliwack River Hatchery which is located in the extreme southern portion of the Fraser River watershed of British Columbia. Coho were reared from egg to fingerling at Cultus Lake Laboratory. Fish of similar size were used in each test, but since the fish grew during the study, mean wet weights increased from 0.38 to 4.90 g. Fish stocks were reared at 7°C, but groups for testing at other temperatures were gradually acclimated to the test temperature

at 1° per day or less and then reared at least 3 wk at the test temperature prior to testing (APHA et al. 1985). The natural photoperiod was followed for rearing and testing.

Testing commenced with swim-up coho fry (0.38 g) in May and concluded with coho fingerlings in December. Ninety-six-hour bioassays of SS were conducted at temperatures ranging from 1 to 18°C, and throughout the period of study at 7°C as a reference. Five experimental SS concentrations and a control (no added sediment) were set up for each 96-h test of lethality. Numbers and sizes of fish in test vessels were as follows: 25 fish (0.45 g or less), 15 fish (0.46–1.00 g), 10 fish (1.01–3.99 g), and 5 fish (4.00–4.90 g). Sixteen bioassays (5 × 16 = 80 SS concentrations) were conducted at the 7.0°C reference temperature. Duplicate bioassays were conducted at 1.0 and 14.0°C (10 SS concentrations at each temperature). Single bioassays were made at 2.0, 3.4, 3.8, 11.4, 16.0, and 18.0°C (five SS concentrations at each temperature). One hundred and thirty SS concentrations were bioassayed of which 104 were suitable for use in concentration – percent mortality plots (log-probit) to derive 96-h LC50 values and 95% confidence limits (Litchfield and Wilcoxon 1949). Some concentrations were not used in the log-probit plots because pump failures allowed sediments to settle. A 96-h LC1 was read from each log-probit plot. The GLM procedure was used to compute analysis of variance, since the 96-h LC50 data set contained unequal replications (SAS Institute Inc. 1985). We compared means using Duncan's multiple range test (SAS Institute Inc. 1985) and the Tukey–Kramer test (Sokal and Rohlf 1981).

The 104 test concentrations used to derive 96-h LC50's were based on 741 samples for SS measurements. These measurements were used to calculate 95% confidence intervals (CI) and CI as a percentage of SS (CI/SS × 100) for each 10 g $SS \cdot L^{-1}$ interval.

Results

The Fraser River sediments used during bioassays with coho were identical to those described by Servizi and Martens (1987), having been drawn from the same well-mixed stockpile. More than 90% of the particles were 5 μm and smaller based on geometric mean diameter (GMD). Analysis of particle size by weight (BWT) yielded a median diameter of 75 μm assuming spherical particles (Fig. 1). Although particles 5 μm and smaller were most numerous, they composed only 1% of the weight.

Measurements indicate low temporal variability of SS concentrations during bioassays. Mean 95% CI's ranged from 0.57 to 2.11 g $SS \cdot L^{-1}$ over the four test ranges (Table 1). Confidence intervals averaged 6% of measured SS concentrations in the 20.1–30.0 $g \cdot L^{-1}$ range. For example, at the 25 g $SS \cdot L^{-1}$ exposure level the 95% CI's are 23.5 and 26.5 $g \cdot L^{-1}$.

No mortalities occurred among control fish in any of the bioassays. However, coincident with the bioassay of SS at 16°C, mortalities occurred among the fish acclimated to this temperature. Subsequent examination indicated that a viral kidney infection was the probable cause of mortalities. The fish acclimated to 16°C were not used in any further tests. No diseases were observed among other stocks of fish used in bioassays, and mortalities were less than 1.0% during acclimation.

Mean length of test fish increased from 3.8 to 7.3 cm over the course of the study (Fig. 2). During May, 96-h LC50's were 8.0 and 8.2 $g \cdot L^{-1}$ at 7.0°C for two bioassays. The 96-h LC50's rose sharply as fish length increased from 4.0 to 4.6 cm and weight increased 40% from 0.52 to 0.73 g. Thereafter, from June through December, tolerance to SS was independent of fish size at 7.0°C and the mean 96-h LC50 was 22.7 $g \cdot L^{-1}$ (SE = ±0.7).

The 96-h LC50 was temperature dependent in the range 1–18°C (Fig. 3). Juvenile coho were most tolerant of SS at 7.0°C where the mean 96-h LC50 was 22.7 $g \cdot L^{-1}$. The 96-h LC50 at 1.0°C was 47% of the mean at 7°C. At 18°C, when fish were confined to the upper 26 cm, 96-h LC50's were 7.0 and 8.1 $g \cdot L^{-1}$, respectively, for SS measured at the surface and at 8 cm depth. The mean of these two values (plotted in Fig. 3) is 33% of the 96-h LC50 at 7°C. The lowest 96-h LC50 (1.3 $g \cdot L^{-1}$) occurred at 16°C. As explained under Methods, the fish used in this bioassay were drawn from a temperature-acclimated stock which was subsequently diagnosed to have a kidney infection. Although logistics precluded disease testing at the time of bioassay, we believe that fish used in the 16°C bioassay were weakened by the infection and were thus less tolerant of SS. For all the 96-h LC50 and temperature data, analysis of variance (GLM) yielded $p = 0.0001$, indicating that a significant difference existed. The 96-h LC50's measured at temperatures above and below 7°C were significantly lower than the mean at 7°C; specifically, $p < 0.05$ at 2, 3.6, and 11.4°C, while $p < 0.01$ at 1, 14, 16, and 18°C.

The 96-h LC50 values serve as useful reference points for acute toxicity. However, managers of fish habitat and water quality also have an interest in maximum SS levels which are unlikely to be acutely lethal. To estimate these levels, maximum SS concentrations at which no mortalities occurred during 96-h tests and 96-h LC1 values were plotted in Fig. 3. The mean SS at 7°C was 8.1 $g \cdot L^{-1}$ for concentrations at which no mortalities occurred ($n = 10$), while the mean 96-h LC1 was 8.2 $g \cdot L^{-1}$ ($n = 14$). These values are 36% of the mean 96-h LC50 at 7°C. A line of best fit by eye was drawn through the 96-h LC1's and "no mortality" data. At 1 and 18°C the no-mortality levels are about 25% of the no-mortality value at 7°C. Suspended solids concentrations below the "no-mortality" line are unlikely to cause acute mortalities among fingerling coho 4.6 cm or larger. To protect smaller coho fry would require even lower levels of SS according to Fig. 2. Based on results at 7°C (Fig. 2) the no-mortality level for smaller coho (0.52 g or less) may be only 35% of the no-mortality value for larger coho, and fish habitat should be managed with this in mind.

Discussion

Fraser River sediments identical to those used in tests of coho were also used in tests with juvenile sockeye (*O. nerka*, Servizi and Martens 1987) and chinook (*O. tshawytscha*, Servizi and Gordon 1990). The 96-h LC50's for sockeye and chinook were 17.6 and 31 $g \cdot L^{-1}$, respectively. The average weights for sockeye were 0.8–1.5 g and tests were conducted at 7.8 and 8.3°C. The chinook were between 1.0 and 7.0 g wet weight and were tested at 7°C. Thus for Fraser River salmon of similar size tested at similar temperatures in identical SS, chinook were most tolerant followed by coho and sockeye.

The tolerance to SS of 0.52-g coho was only 35% that of the larger fish. The lower tolerance of the smaller fish may be related to capacity to clear the buccal cavity of sediment particles, as discussed below. Observations indicate that coho increase their rate of gill flaring when exposed to SS (Berg and Northcote 1985). Gill flaring abruptly changes buccal cavity pressure and is a means of clearing the buccal cavity of foreign

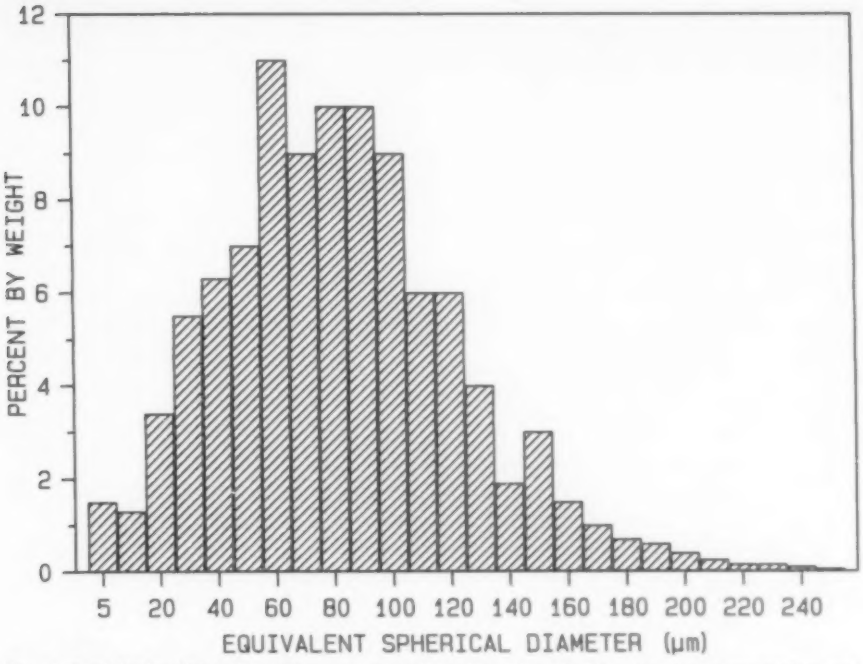

FIG. 1. Particle size distribution of Fraser River sediments (Ft. Langley) based on the bottom withdrawal tube method.

TABLE 1. Confidence intervals (CI) for suspended sediment (SS) measurements in test vessels.

SS range ($g \cdot L^{-1}$)	SS concentrations tested (number)	SS measurements		
		Number	Mean 95% CI ($g \cdot L^{-1}$)	Mean CI/SS × 100 (%)
1.0–10.0	29	208	0.57	9
10.1–20.0	35	241	0.98	7
20.1–30.0	27	203	1.39	6
30.1–40.0	13	89	2.11	6
Total	104	741		

material (Heath 1987). McLeay et al. (1987) observed increased coughing among Arctic grayling (*Thymallus arcticus*) exposed to SS. Servizi and Gordon (1990) postulated that sediments accumulated in buccal cavities when fish became too fatigued to continue clearing particles via the cough reflex. In another study, tolerance of fingerling sockeye to SS decreased with increasing particle size (Servizi and Martens 1987). Presumably this result occurred because a stronger cough reflex would be required to clear the larger particles from the buccal cavity. Taken together, the foregoing observations suggest that strength of the cough reflex may be a factor in SS tolerance. If this is the case, then the 0.52-g coho may have been less tolerant than the 0.73-g fish because the latter were able to create sufficient water velocity in the buccal cavity to clear sediments via the cough reflex. The low tolerance of small salmon may have implications for emergent fry. For example, emergent sockeye weigh about 0.2 g. The tolerance of fry to SS is a subject deserving further research.

Suspended sediments also lower resistance to disease. Redding et al. (1987) believed that stress associated with exposure to SS was responsible for lowered resistance to a bacterial pathogen among test groups of yearling steelhead (*O. mykiss*). The reverse may also occur; disease may lower tolerance to SS as seen at 16°C in Fig. 3. In this case, we believe that SS tolerance was reduced (approximately 85%) by an infection which was not outwardly evident during the test. The combined stresses caused by SS and infection evidently compounded to lower tolerance of the fish.

Noggle (1978) suggested that temperature may be a major factor in seasonal tolerance to SS by coho. Our results (Fig. 3) indicate that coho tolerance to SS is indeed temperature dependent with maximum tolerance near 7°C and declining tolerance above 7°C. A parallel phenomenon was observed in tests of three estuarine species. Tolerance to SS decreased for fourspine stickleback (*Apeltes quadracus*), cunner (*Tautogolabrus adspersus*), and sheepshead minnow (*Cyprinodon variegatus*) in the temperature ranges 9–16, 15–25, and 10–19°C, respectively (Rogers 1969).

Tolerance to SS may be the net result of a combination of physical and physiological factors related to oxygen availability and uptake by fish. Impaired oxygen exchange at the gill surfaces was believed to be the primary cause of death among sockeye and chinook exposed to volcanic ash (Newcomb and Flagg 1983). Noggle (1978) and Rogers (1969) also attributed acute mortality in SS mixtures to reduced oxygen uptake. Oxygen transfer at gill surfaces, oxygen content of the water, metabolic oxygen demands, and capacity to perform work are likely to be major factors in tolerance to SS. To keep gills clear for oxygen exchange requires the fish to perform work in the form of cough reflex. When physiological and environmental conditions combine to limit the capacity to clear the gills, fish will succumb to SS. Cruising speed is a measure of the capacity to sustain work and provides an indication of a fish's ability to maintain cough frequency and ventilation rate. Brett (1958)

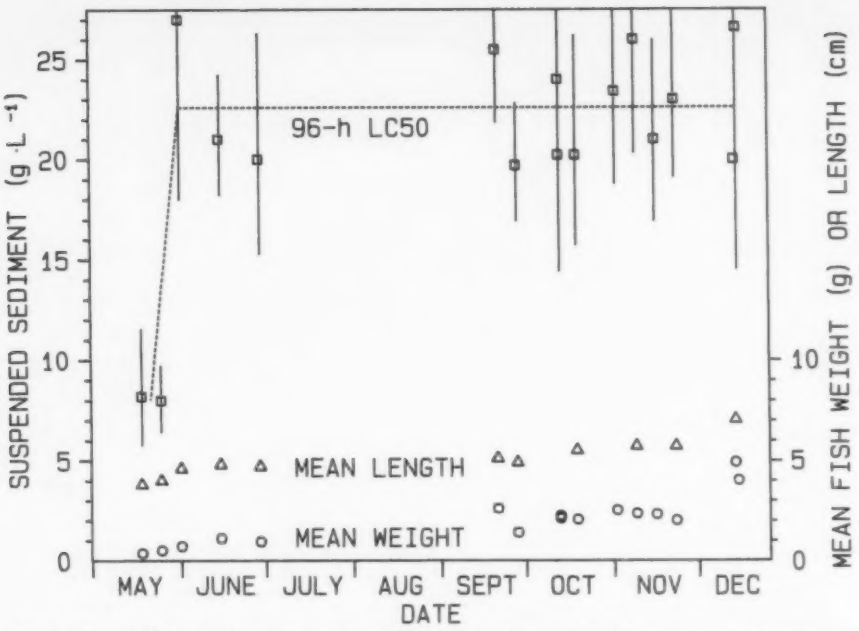

FIG. 2. Mean weights and lengths of underyearling coho and acute lethalities of suspended sediments (96-h LC50) at 7°C. Vertical lines indicate 95% confidence limits.

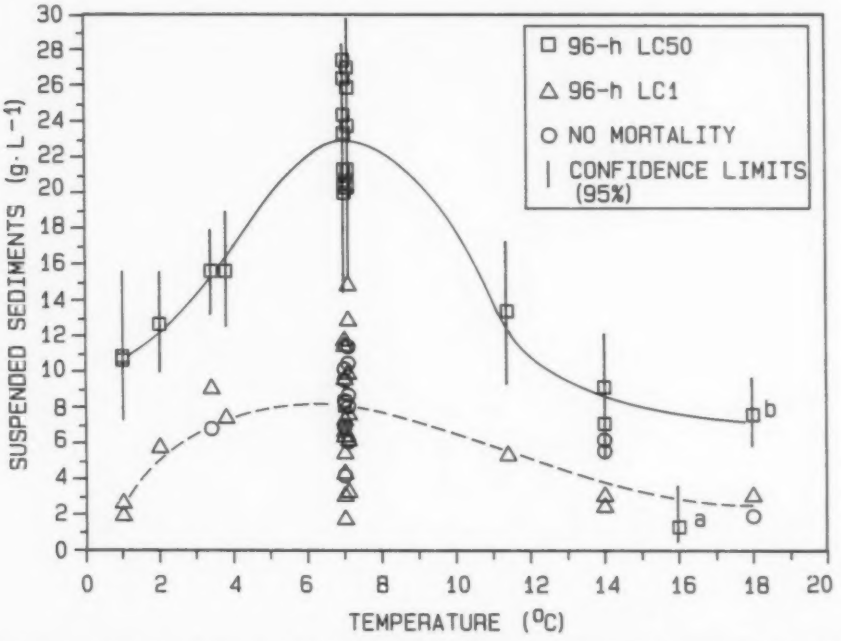

FIG. 3. Tolerance of underyearling coho to suspended sediments at temperatures between 1 and 18°C. a, fish were stressed (see text); b, fish were partially confined (see text).

found that cruising speed in underyearling coho increased from 18 to 30 $cm \cdot s^{-1}$ while temperature increased from 7 to 18°C. Although this 67% increase in ability to perform work seems to favour a fish's ability to tolerate SS, the energy to perform repeated coughing increases metabolic oxygen demand. Furthermore, metabolic oxygen demand increases with temperature (Brett 1964). For example, at rest (standard metabolism) the oxygen consumption rate for 50-g sockeye calculated from Brett (1964) increased from 45 to 93 $mg \cdot kg^{-1} \cdot h^{-1}$ while temperature increased from 7 to 18°C. While sockeye and coho may not have identical standard metabolic oxygen demands, it is reasonable to assume a proportionally similar increase for

coho over the same temperature range. In addition to the foregoing, as water temperature increases from 7 to 18°C, oxygen saturation decreases 22%. Thus as temperature increases from 7 to 18°C, fish have increased capacity to perform work, but standard metabolic demand for oxygen increases approximately 100% and oxygen in the water decreases 22%. In the case of coho exposed to SS, the net effect of temperature increase from 7 to 18°C is a 67% reduction in tolerance to SS.

At low temperatures, oxygen saturation and standard metabolic oxygen demand favour a fish's tolerance to SS. For example, the oxygen saturation value for water increases 17% as temperature decreases from 7° to 1°C while the standard metabolic oxygen demand decreases 13%. However, reduced tolerance to SS at low temperatures may be primarily related to capacity of the fish to perform work associated with maintaining the cough reflex and ventilation rates that are adequate to clear the gills of particles. That is, survival would depend on the capacity to do adequate work at low temperature. The cruising speed of 5.4-cm coho declined from 18 to 7 $cm \cdot s^{-1}$ as acclimation temperature decreased from 7 to 1°C (Brett et al. 1958). This 61% decrease is an indication that coho have a much reduced capacity for work at 1°C, and this may be a major reason for lower tolerance to SS at low temperature.

In summary, our results indicate that the relationship between SS tolerance and temperature probably involves several factors: cough reflex, oxygen transfer, oxygen saturation levels, metabolic rates, and capacity to do work. The data also indicate that salmonid fry are likely to be less tolerant of SS than fingerlings. Judging from studies with sockeye (Servizi and Martens 1987), tolerance is also influenced by particle size and thus caution is warranted when tolerance data obtained using one sediment are applied to another with a different particle size frequency. Finally, since particle size is an important factor in SS tolerance, it is recommended that investigations specify whether particle size frequency is expressed in terms of weight or in number of particles because the two methods are not equivalent, as shown herein for Fraser River sediment.

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Lake Trout (*Salvelinus namaycush*) Control of Snail Density and Size Distribution in an Arctic Lake

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In arctic Alaskan lakes the density and size distribution of the snail *Lymnaea elodes* appear to be greatly affected by the presence or absence of lake trout (*Salvelinus namaycush*). Snail densities were much lower and size distributions depressed in lakes where trout were present. During the summer of 1986, lake trout were introduced into an arctic lake to test the effects of their predation on the dominant snail *Lymnaea elodes*. During July and early August, snail densities in the experimental lake remained stable in the deepwater areas of the lake, but increased significantly in the deepwater areas of an adjacent, troutless control lake, probably due to snail migration from the lake periphery in the absence of predation pressure. These experimental results suggest that the observed pattern of snail distribution and size in lakes containing trout is due to lake trout predation.

Dans les lacs arctiques de l'Alaska, la densité et la répartition des lymnées *Lymnaes elodes* par catégorie de dimensions semblent grandement influencés par la présence ou par l'absence de la truite de lac (*Salvelinus namaycush*). Dans les lacs où la truite était présente, les densités des lymnées étaient beaucoup plus faibles et les distributions des catégories de dimensions étaient à la baisse. Au cours de l'été 1986, on a introduit la truite de lac dans un lac arctique pour tester les effets de la prédation des truites sur la principale lymnée qui s'y trouvait (*Lymnaea elodes*). En juillet et au début d'août, les densités des lymnées du lac expérimental sont restées stables dans les zones profondes du lac, mais ont augmenté considérablement dans les zones profondes d'un lac témoin adjacent d'où la truite était absente, probablement à cause de la migration des lymnées de la périphérie vers les zones profondes, en l'absence de prédation. D'après les résultats de cette expérience, le profil qui se dégage de la distribution et des dimensions des lymnées dans les lacs où vit la truite pourrait être imputable à la prédation par la truite de lac.

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The introduction of a fish species into a lake can provide an opportunity to study the role fish predation plays in shaping community structure (Nilsson 1972). Predation by fish on benthic invertebrates is not necessarily related to size of the prey item, but may be influenced by factors such as activity, exposure, and density (Ware 1973; Crowder and Cooper 1982; Werner et al. 1983; Hershey 1985). Macan (1965, 1966, 1977) noted that less abundant, but conspicuous, prey species were more strongly influenced by the introduction of brown trout (*Salmo trutta*). Brown trout were observed to eliminate or limit the distribution of such prey species. Habitat complexity alters the effects of predation by providing refuge for prey species and reducing fish foraging efficiency (Crowder and Cooper 1982; Gilinsky 1984; Hershey 1985).

Thorp (1986), in a review of the effects of predators on freshwater communities, assigned predation one of two, often mutually exclusive roles in shaping freshwater communities: (1) some predators may significantly influence diversity of present-day communities by interrupting processes of competitive exclusion; (2) other predators may have an important historical role in determining the composition of the current species pool, but only a minor role in modern communities.

In the Toolik Lake region of arctic Alaska, the pulmonate snail *Lymnaea elodes* is the major prey item in the diet of lake trout (*Salvelinus namaycush*). *Lymnaea* has been shown to be the dominant competitor in gastropod communities (Brown 1982; Brown and DeVries 1985; Hershey 1990). In the Toolik Lake region, *Lymnaea* dominates competitive interactions with the snail *Valvata lewisi* (Hershey 1990). In addition, *Lymnaea* populations have been shown to be limited by intraspecific competition (Eisenberg 1966; Brown 1982; Cuker 1983). Round whitefish (*Prosopium cylindraceum*) also prey on *Lymnaea*, but are limited to snails of juvenile size. In lakes where lake trout are absent, *Lymnaea* is 59-fold more abundant on soft sediments than when trout are present and reaches a larger adult size (Hershey 1990).

To test the hypothesis that lake trout predation determines *Lymnaea* densities and size distributions, we introduced a population of trout into a small, troutless arctic lake (Lake S7) in the summer of 1986. We examined the effects of trout foraging on the deepwater density and size distribution of *Lymnaea* in Lake S7 compared with Lake S6, a small neighboring lake that

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lacked trout and had a similar *Lymnaea* population. We also examined the diets of fish in Toolik Lake and the nearshore density and size distribution of *Lymnaea* in lakes containing lake trout and compared them with the density and size distribution of *Lymnaea* in lakes without lake trout.

Study Area

The Toolik Lake area on the north slope of the Alaskan Brooks Range is located approximately 216 km inland from Prudhoe Bay. Lakes in this region are highly oligotrophic and ice-free from late June until late September or early October (e.g. O'Brien et al. 1979; McDonald et al. 1982; Hershey 1985; Miller et al. 1986). Lakes with a depth ≥3 m support some combination of five fish species: lake trout, slimy sculpin (*Cottus cognatus*), burbot (*Lota lota*), round whitefish, and Arctic grayling (*Thymallus arcticus*).

This paper focuses on a lake trout introduction experiment involving two small lakes 0.5 km west of Toolik Lake. Lake S6 (2.8 ha) and Lake S7 (3.6 ha) are small glacial kettle lakes with maximum depths of 7 and 4.5 m respectively. The fish species present in these two lakes include Arctic grayling and slimy sculpin. One juvenile burbot has been captured in Lake S6 and none have been sampled in Lake S7. Burbot and sculpin captured in Toolik Lake have not been observed to eat snails (Hershey 1985). It is not known whether fish can overwinter in the shallower Lake S7, but they may overwinter in Lake S6 and recolonize Lake S7 each spring by migrating through the short creek connecting the two lakes. *Lymnaea elodes* is the dominant mollusc in these lakes. All sizes of *Lymnaea* were present throughout the summer, indicating that these snails live more than 1 yr and are capable of overwintering (Hershey 1990). Other prominent invertebrates are *Gerensia* (Trichoptera), *Valvata lewisi* (Gastropoda), Sphaeriid clams, and chironomids. Substrates in Lake S7 are primarily soft with small patches of sparse macrophytes and some rocky substrate near the small outlet which flows into Lake S6. Lake S6 is rocky near shore and has deeper soft substrates similar to those found in Lake S7. On the open sediments in these lakes, adult *Lymnaea* were easily observable due to their large size.

Methods

Nearshore *Lymnaea* density and size distribution were compared between three lakes with lake trout (Toolik, I8, and N1) and three lakes without lake trout (S7, S6, and N2). Sampling was conducted over open sediments in less than 1 m of water. In each lake, a sampling frame (2.0 m^2) was haphazardly tossed from shore three times in areas of soft sediments. All *Lymnaea* were collected, counted, measured with vernier calipers to the nearest 0.1 mm, and released in the area of capture. Lakes S6 and S7 were sampled on 1 July 1986, lakes N1 and N2 on 9 July 1986, lakes I8 and Toolik on 1 August 1986. Since *Lymnaea* are slow growing and have a multiyear life cycle in this system, we did not expect that differences in sampling date would have a significant effect on density or size estimates. Statistical comparisons were made with *t*-tests for independent means. Means are reported ±1 SE for this and all other comparisons. Satterthwaite's approximation for degrees of freedom was used due to unequal variances (SAS Institute Inc. 1985).

Fish for diet analysis were collected from Toolik Lake with an experimental gill net (38.1 m long, 2 m deep; five panels: 19, 25, 38, 51, and 64 mm). Fish stomachs were preserved in ethanol. All stomach contents from lake trout, round whitefish, and grayling were sorted and enumerated, and the lengths (longest shell axis) of all molluscs were measured using a Nikon Micro-Plan II digitizing pad. To compare prey abundances among fish species the mean number of each prey type in fish stomachs was compared using Tukey's studentized range test for minimum significant difference (SAS Institute Inc. 1985).

To determine if lake trout and round whitefish selected snails by size the mean prey size available on the rocks (*Lymnaea*) and soft sediments (*Valvata* and clams) of Toolik Lake was compared with mean mollusc size eaten by *t*-tests for independent means. *Lymnaea* from nearshore samples described previously were used in comparisons with the mean size of *Lymnaea* eaten by lake trout and round whitefish. *Valvata* and clams were sampled on the open-sediment littoral zone (5.0 m depth) of Toolik Lake on 8 August 1982 (Hershey 1985). Using SCUBA, eight samples were taken with a hand-held, 10-cm-diameter plastic core tube (78.54 cm^2). The mean size of molluscs eaten was also compared between round whitefish and lake trout with *t*-tests for independent means. In these comparisons, degrees of freedom were determined with Satterthwaite's approximation to account for unequal variances (SAS Institute Inc. 1985).

We began introducing lake trout into Lake S7 on 29 June 1986. Fish were captured in Toolik Lake with an experimental gill net and by angling. Fish were measured, weighed, and given an identification mark by clipping a single dorsal and anal fin ray in unique combinations. This marking technique left small recognizable scars that did not appear to affect the swimming ability of the fish. Uninjured fish were transported in buckets to a holding area near the outlet of Lake S7. The outlet of Lake S7 was blocked with a small rock dam prior to the introduction to prevent fish from escaping. A total of 25 trout were released during the course of the experiment (29 June to 4 August), but most fish were not present throughout the experimental interval. The average number of lake trout present in Lake S7 through the 36 d of the experiment was 14, which translates into an average density of 3.9 trout/ha. This is within the range of 1–14 trout/ha reported from arctic Canadian lakes (Martin and Olver 1980). The effective density of lake trout in Lake S7 was probably closer to 8 trout/ha due to the shallow nature (<1 m) of one of its two basins. Trout were not observed to use the shallow basin. Mean total length of the trout was 416.4 ± 12.5 mm (mean ± SD) with a maximum of 585 mm and a minimum of 273 mm. This introduced population was not significantly different from the sampled size distribution of lake trout in Toolik Lake ($t = 0.436$, df = 58, $p < 0.5$).

Offshore *Lymnaea* density and size distribution data were determined by swimming four 10-m transects using SCUBA in both the experimental lake (S7) and the control lake (S6) on three dates (3 July, 26 July, and 6 August).

All *Lymnaea* within an arm's length of each transect were collected. An area of 10 m^2 was sampled at each of the four sites in both lakes on each date. The transects covered depths of approximately 3–4.5 m in Lake S7 and 3–6 m in Lake S6. Snails were brought to shore, counted, and measured to the nearest 0.1 mm with vernier calipers and then released in the area of capture.

Nearshore *Lymnaea* density data were collected by harvesting all *Lymnaea* within a 2-m^2 sampling frame placed on the substrate at 10 sites in Lake S7 and six sites in Lake S6 on three dates (3 July, 26 July, and 4 August). The sampled sites were in approximately 1 m of water in both lakes. Sampled snails

TABLE 1. Mean (± SE) number of prey items in the stomachs of lake trout (n = 74), round whitefish (n = 71), and Arctic grayling (n = 10) from Toolik Lake. Two asterisks indicate significant difference between indicated mean and both compared means (Tukey's msd test, $P < 0.05$).

Prey taxon	Lake trout	Round whitefish	Arctic grayling
Lymnaea	20.8 ± 3.9**	6.4 ± 1.8	0.1 ± 0.1
Valvata	86.3 ± 69.3	40.5 ± 13.0	2.6 ± 1.0
Clams	10.8 ± 4.5	13.1 ± 3.9	—
Total fish	0.3 ± 0.07	—	—
Slimy sculpin	0.2 ± 0.06	—	—
Caddis	3.2 ± 1.6	27.8 ± 5.9**	9.8 ± 4.5
Chironomids	17.5 ± 4.5	12.3 ± 5.1	2.8 ± 2.2
Zooplankton	135.2 ± 49.7	—	298 ± 175.5

were counted, measured, and released similar to those collected in offshore samples.

To test for significant differences in *Lymnaea* density between lakes and with regard to lake × date interactions, a split plot with repeated measures ANOVA was used. Lakes S6 and S7 were each regarded as plots, and samples from each of four sites within a plot were taken four times. Prior to analysis, variances were homogenized with a ln (x + 1) transformation (Sokal and Rohlf 1981). To test for significant changes in snail densities over time within a lake, a one-way ANOVA with repeated measures was used. Tukey's studentized range test was used to calculate the minimum significant difference (SAS Institute Inc. 1985). The mean sizes of *Lymnaea* were analyzed for significant differences between lakes S6 and S7 with a t-test for independent means with Satterthwaite's approximation for degrees of freedom due to unequal variances on each date.

From 8 August to 10 August, following the final transect sampling date, an attempt was made to recapture the introduced lake trout. A 100-ft (1 ft = 0.3048 m) experimental gill net was used to bisect the deep basin and approximately half of the littoral region on each side of Lake S7. We identified, weighed, measured, and sexed each recaptured fish. Stomachs were removed and preserved for later analysis. All organisms were identified and enumerated. Molluscs were measured with a Nikon Micro-Plan II digitizing pad. *Lymnaea* were measured to the nearest 0.1 mm and all other molluscs were measured to the nearest 0.01 mm.

Results

Support for the hypothesis that trout control snail density is found in a lake survey of nearshore *Lymnaea* densities. In lakes with lake trout, *Lymnaea* were found in significantly reduced densities ($\bar{x}$ = 0.4 ± 0.2 snails/m^2; n = 3) when compared with lakes without lake trout ($\bar{x}$ = 8.9 ± 0.8 snails/m^2; n = 3, t = 10.006, df = 9, $p < 0.00005$). In addition, the mean size of adult snails in troutless lakes (20.6 ± 0.4 mm) was significantly larger than the mean size from lakes with trout (15.3 ± 0.3 mm; t = 9.941, df = 31, $p < 0.00005$).

Lake trout in Toolik Lake ate significantly more *Lymnaea* than round whitefish and grayling (Table 1). Compared with round whitefish, lake trout in Toolik Lake ate significantly larger *Lymnaea* (t = 21.739, df = 414, $p < 0.00005$; Table 2). Lake trout also ate significantly larger *Lymnaea* than the mean size available on the rocks of Toolik Lake (t = 9.024, df = 38, $p < 0.00005$; Table 2).

TABLE 2. Comparison of mean (± SE) size of molluscs from lake trout and round whitefish stomachs and sampled from substrates in Toolik Lake. One asterisk indicates significant difference between mollusc size selected by lake trout versus round whitefish. Two asterisks indicate significant difference between mollusc size selected by the indicated fish species and size of the molluscs sampled on the substrate.

	Fish stomachs		Benthic samples	
Prey taxon	Lake trout	Round whitefish	Sediment	Rocks
Lymnaea	13.0 ± 0.17*,**	7.0 ± 0.21	—	5.6 ± 0.8
Valvata	4.5 ± 0.04*,**	3.6 ± 0.05**	2.9 ± 0.12	—
Clams	5.2 ± 0.09*,**	3.3 ± 0.05**	2.6 ± 0.13	—

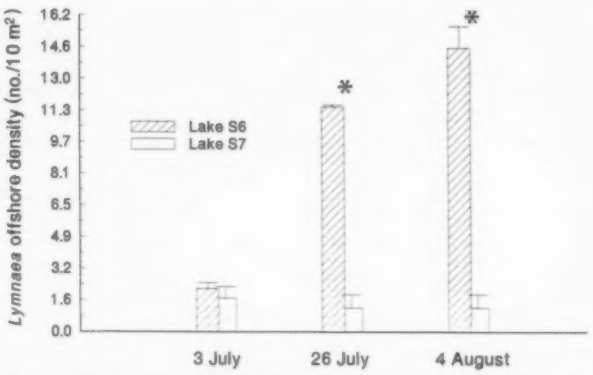

FIG. 1. Offshore *Lymnaea* density (mean ± SE) over three dates in Lake S6 (control) and Lake S7 (lake trout introduction). Asterisks indicate significantly different *Lymnaea* densities between lakes at $p < 0.05$.

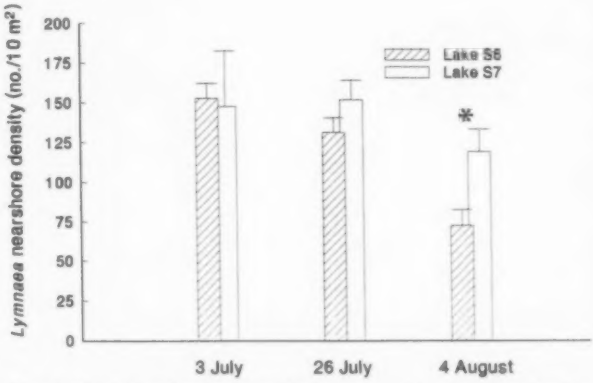

FIG. 2. Nearshore *Lymnaea* density (mean ± SE) over three dates in Lake S6 (control) and Lake S7 (lake trout introduction). Asterisk indicates significantly different *Lymnaea* densities between lakes at $p < 0.05$.

Deepwater densities of *Lymnaea* in the control lake (S6) and the experimental lake (S7) diverged significantly through the course of the experiment. Initial sampling on 3 July yielded snail densities for the control and experimental lakes that were nearly identical, but on subsequent sampling dates, *Lymnaea* densities were significantly higher in Lake S6 (Tukey's studentized range test: msd = 1.25, df = 18, $p < 0.05$ for both 26 July and 4 August; Fig. 1). In addition, a significant lake × date interaction (F = 4.95, df = [1,3], $p < 0.027$) implies

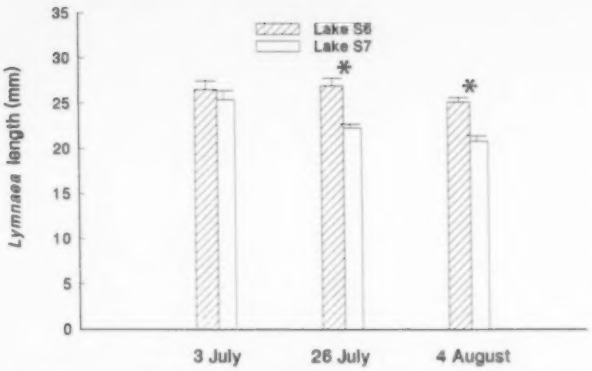

FIG. 3. *Lymnaea* size distribution (mean ± SE) over three dates in Lake S6 (control) and Lake S7 (lake trout introduction). Asterisks indicate significantly different *Lymnaea* size distributions between lakes at $p < 0.01$.

that snail densities in the two lakes diverged over time (Fig. 1). Looking at each lake independently shows that offshore snail densities in the experimental lake (trout introduction) did not change significantly over the three sampling dates ($F = 0.11$, df = [2,3], $p < 0.9$; Fig. 1). In the control lake, however, offshore snail densities significantly increased ($F = 5.81$, df = [2,3], $p < 0.04$; Fig. 1). Initial and final densities were significantly different (Tukey's studentized range test: msd = 11.498, df = 6, $p < 0.05$).

A decline in nearshore *Lymnaea* densities occurred in Lake S7 as the summer progressed (Tukey's studentized range test: msd = 6.76, df = 27, $p < 0.05$; Fig. 2). Lake S6 showed a similar trend toward seasonally declining *Lymnaea* density, but it was not significant (Fig. 2).

Coupled with reduced densities on deepwater sediments in the experimental lake was a significant suppression in the size distribution of *Lymnaea* (Fig. 3). Initial sampling on 3 July produced size distributions that were not significantly different ($t = 0.79$, df = 14, $p < 0.02$). On the two later sampling dates, offshore *Lymnaea* size distributions were significantly smaller in Lake S7 (26 July, $t = 2.91$, df = 49, $p < 0.01$; 4 August, $t = 2.99$, df = 60, $p < 0.005$; Fig. 3).

Observations of lake trout by SCUBA and snorkeling and observing fish tagged with balloons on 25-ft monofilament leads lend support to the hypothesis that the introduced fish were using and feeding in the deepest areas of the lake. On three occasions while diving or snorkeling, a total of five fish were observed swimming over deep water (>3 m). Only two observations were made of fish using the littoral region of the lake, and in both instances the fish appeared to be unhealthy. Observations of three lake trout tagged with balloons were made on three consecutive days in early August. Two of these three fish were never observed in shallow water subsequent to 1 d after release and appeared to spend most of their time slowly cruising outside the deep edge of the breakline (approximately 3 m). The third fish appeared unhealthy and disoriented.

Five fish were recaptured by gill net from Lake S7. One additional fish was recaptured by angling. Gut analysis showed that only two of the six fish had recently consumed *Lymnaea*; the remaining four had empty guts. All fish recaptured by gillnetting were caught deep in the net (depth = 3.5–4.0 m) over the deepest portion of the lake.

Discussion

Experiments concerning the role of predators in freshwater systems have most often been done in communities that are relatively complex when compared with the lakes in the Toolik region (e.g. Thorp and Bergey 1981a, 1981b; Crowder and Cooper 1982; Gilinsky 1984). The relative simplicity of the fish and benthic communities in the Toolik area lakes affords the opportunity to investigate the role of lake trout in influencing a simplified benthic community. Lake trout – *Lymnaea* interactions in Toolik area lakes do not appear to operate under the constraints suggested by Thorp and Bergey (1981a), but rather, may be representative of predator–prey interactions in communities with low diversity.

In our experiment, we introduced a natural density of a predator into a lake with an unexploited prey population. Without other significant predators present, we could be reasonably sure that the changes we detected in the *Lymnaea* population were due to trout predation. Observations of lake trout by SCUBA and snorkeling and observing tagged fish support this conclusion, since trout were usually sighted in deep water (≥3.0 m). In addition, all five lake trout recaptured by gillnetting were located deep in the gill net over the deepest available water in Lake S7. Two of the six lake trout recaptured had recently consumed *Lymnaea*. Although one trout consumed only one small *Lymnaea* (9.4 mm), the other consumed 17 Lymnaea (16.7 ± 0.5 mm). This final observation is not sufficient to demonstrate that predation on snails was great enough to explain the observed reduction in size distribution between Lake S7 and Lake S6, but it does provide evidence that the introduced lake trout were actively feeding on large, reproductively sized (>15 mm, see Hershey 1990) *Lymnaea* during the experiment.

The apparent migration of *Lymnaea* from the shallow to the deep sediments during the summer in Lake S6 was an unexpected result which needs further investigation. High levels of intraspecific competition in *Lymnaea* populations have been observed previously (Eisenberg 1966; Brown 1982; Cuker 1983); thus, food limitation on the shallow sediments is a plausible mechanism for offshore migrations. In Toolik Lake, *Lymnaea* significantly reduces benthic algal standing crops and its growth rate is limited by intraspecific density (Cuker 1983). The observed seasonal increase in *Lymnaea* on offshore sediments in Lake S6 but no increase in Lake S7 may be due to predation by lake trout on migrating snails. The migration hypothesis is also consistent with the apparent gradual decline in nearshore density during the summer, especially in Lake S7. However, in lakes with lake trout and greatly reduced *Lymnaea* densities, *Lymnaea* should not become as food limited, and therefore, may not migrate offshore during the summer. Thus, an alternative explanation for the observed pattern of very low density of *Lymnaea* in trout lakes (Hershey 1990 and Fig. 1) may be that snails remain nearshore to minimize their exposure to predators. Offshore *Lymnaea* densities reported by Hershey (1990) in lakes without lake trout are similar to those found in Lake S6 and provide preliminary evidence that the migration occurs in other troutless lakes in the Toolik area.

Nearshore densities reported here indicate lower density in lake trout lakes, but are potentially confounded by a hypothesized migration. If migration occurs, and it has a significant effect on nearshore densities, then the sampling survey data of nearshore *Lymnaea* density between lake trout and no lake trout lakes need to be interpreted with caution. Sampling in two of the trout lakes was conducted in August when nearshore density

would be minimal if snails migrated, and sampling in two no lake trout lakes was conducted on 1 July when nearshore density would be maximal; thus, differences would be maximized between groups of lakes. One of two scenarios applies: (1) snails migrate offshore during the summer in lake trout lakes, and since their offshore density is essentially zero (Hershey 1990 and Fig. 1), they are eaten by trout as they migrate or (2) snails remain near shore to minimize their exposure to predators. If snails did not migrate, the timing of the sampling, which shows fewer snails in lake trout lakes, would make no difference.

Predation by lake trout on *Lymnaea* in Toolik area lakes may play a major role in the causation and maintainence of gastropod diversity, since *Lymnaea* competitively controls *Valvata* density in the absence of trout (Hershey 1990). *Valvata* produce more offspring when *Lymnaea* are absent regardless of resource availability and occur at higher densities in lakes where *Lymnaea* are absent from the open sediments (Hershey 1990). Thorp (1986) stated that a predator that determines prey diversity does so by affecting the kinds of species that can exist in an environment for which the prey taxa are otherwise suited. *Lymnaea* are clearly suited for the open sediments of lakes in the Toolik Lake region, but are excluded from them by lake trout predation. Lake trout prey on *Lymnaea* more frequently, in larger numbers, and on larger individuals than any other fish species in Toolik Lake and in at least two other area lakes (Table 1 and Merrick 1989). Gut data, combined with the experimental and sampling data presented here, suggest that lake trout are responsible for the occurrence of significantly lower *Lymnaea* densities and suppressed size distributions in Toolik area lakes with lake trout when compared with those without trout. Although lake trout clearly eat *Valvata* in addition to *Lymnaea*, persistence of high density of *Valvata* in lakes with trout suggests that lake trout are not directly having a strong impact on *Vavata* populations. Laboratory and field experiments (Hershey 1990) suggest that such a reduction in the competitively dominant *Lymnaea* population would interrupt processes of competitive exclusion and allow the fugitive species, *Valvata*, to attain higher densities. The duration of this trout introduction experiment, however, was too short (approximately 6 wk) to reasonably expect such effects.

The reduction in *Lymnaea* density on the open sediments of Lake S7 is consistent with the hypothesis that conspicuous prey species are most strongly affected by predators (Macan 1966; Ware 1973; Crowder and Cooper 1982; Gilinsky 1984; Hershey 1985). *Lymnaea* on open sediments are conspicuous and vulnerable to predation as is evidenced by their absence from the open sediments in Toolik area lakes that have lake trout. Thus, we conclude that lake trout serve to alter patterns of relative gastropod abundance in these arctic lakes by determining the distribution, abundance, and size distribution of *Lymnaea elodes*, a competitively dominant prey species (Cuker 1983; Hershey 1990). As evidenced by the distribution of *Lymnaea* in trout versus no trout lakes, these effects are not transient (sensu Thorp 1986).

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Effect of Temperature and Size on Development, Mortality, and Survival Rates of the Pelagic Early Life History Stages of Marine Fish

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Pepin, P. 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Can. J. Fish. Aquat. Sci. 48: 503–518.

This paper presents a comprehensive summary of development, mortality, and survival rates of fish eggs, yolk-sac larvae, and postlavae in relation to temperature and size. The general temperature- and, where applicable, size-dependent models explained 30–81% of the variance in vital rates. Daily development and mortality rates generally increased with increasing temperature. Temperature had an equal but opposite effect on stage-specific cumulative mortality rates of eggs and yolk-sac larvae. Stage-specific (length-dependent) cumulative mortality rates of postlarval fish were independent of temperature due to equal effects of temperature on daily growth and daily mortality rates. Size had no significant effect on daily or cumulative mortality rates of egg and yolk-sac stages. Daily mortality rates of postlarval fish were significantly influenced by mean size (i.e. length). The compensation of temperature effects between stages and/or vital rates may reduce the impact of environmental uncertainty on survival rates. It is suggested that the general temperature- and/or size-dependent relationships described in this study can serve as basic frameworks to assess whether there are significant effects resulting from species/stock-specific characteristics or environmental factors on the vital rates of the early life history stages of fish.

Ce rapport présente un résumé détaillé et complet des taux de développement, de mortalité et de survie des oeufs de poissons, des larves en sacs vitellins et des poissons des stades postlarvaires, par rapport aux dimensions et à la température. La température générale et, lorsqu'il y a lieu, les modèles par dimensions étaient à l'origine de 30 à 81 % de la variation observée dans les taux démographiques. Les taux quotidiens de développement et de mortalité augmentaient généralement en fonction de l'augmentation de la température. La température avait un effet égal mais opposé sur les taux cumulatifs de mortalité des oeufs et des larves en sacs vitellins, pour le stade de développement considéré. Les taux cumulatifs de mortalité des poissons des stades postlarvaires, pour un stade donné (suivant la longueur des poissons), étaient indépendants de la température à cause des effets égaux de la température sur les taux quotidiens de croissance et sur les taux quotidiens de mortalité. Les dimensions n'avaient pas d'effet appréciable sur les taux de mortalité quotidiens ou cumulatifs des oeufs et des larves en sacs vitellins. Les taux de mortalité quotidiens des poissons des stades postlarvaires étaient considérablement influencés par les dimensions moyennes (c.-à-d. par la longueur). La compensation des effets de la température d'un stade à l'autre ou d'un taux dæmographique à l'autre peut réduire l'impact de l'incertitude environnementale sur les taux de survie. On suggère que les rapports généraux suivant les dimensions et la température décrits dans cette étude peuvent servir de cadres de base pour déterminer s'il y a des effets appréciables liés aux caractéristiques de l'espèce ou d'un stock de poissons donné ou liés aux facteurs environnementaux sur les taux démographiques des premiers stades de vie des poissons.

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Extensive studies of the development and mortality of fish eggs, yolk-sac larvae, and postlarvae have been conducted (Blaxter 1974; Lasker and Sherman 1981; Blaxter et al. 1989) as a result of Hjort's (1914) hypothesis that recruitment variability is linked to events or factors that influence the vital rates during the early life history stages. It is evident that during this phase of the life cycle, development and mortality rates are high and variable (Dalhberg 1979; Theilacker and Dorsey 1980; McGurk 1986). Therefore, slight variations in the vital rates may lead to significant changes in survival (Houde 1987; Beyer 1989) and consequently recruitment.

To better formalize this concept, consider that the cumulative mortality (C) of a population from one stage or size (a) to another (b) is a direct function of the instantaneous development/growth ($g[x]$) and mortality ($M[x]$) rates such that

$$(1) \quad C = \int_a^b \frac{M[x]}{g[x]}\, dx$$

where x are factors that influence the vital rates. As the cumulative mortality rate increases, the potential sources of variability also increase. The stage during which C is highest is likely to be crucial to reproductive success and would therefore represent the critical period (sensu Hjort 1914). The time (t) required to grow or develop from a to b (Beyer 1989) is

$$(2) \quad t = \int_a^b \frac{dx}{g[x]}$$

and the stage-specific survivorship (Sickle 1977) is

$$(3) \quad \ell(a, b) = \frac{N_b}{N_a} = \exp - \left(\int_a^b \frac{M[x]}{g[x]} dx \right) = \exp - (Mt) = \exp - (C).$$

If one is able to determine the effect of factor(s) x on either development or mortality rates, one should have some ability to predict survival through the different development stages. Houde (1987) and Beyer (1989) have shown that subtle variations in either $g[x]$ or $M[x]$ can lead to large variations in survivorship. A comparison of the results from several studies can yield information on the response of vital rates to specific factors. The first step of such a comparison involves determining the influence of dominant or universal factors on vital rates and trophic interactions.

There is clear evidence that in marine ecosystems, temperature is a key determinant of the rate of energy transfer between trophic levels (e.g. Kemp and Boynton 1981; Livingston 1984; Clarke 1985) and that the size of an organism determines the size and average abundance of prey and predators with which that organism interacts (e.g. Sheldon et al. 1972; Kerr 1974; Silvert and Platt 1978; Pearre 1986). Both temperature and organism size are features that are consistently measured in studies of the early life history of fish. Their effect on some elements of the early life history have been studied by Houde (1989, 1990) and Miller et al. (1988). Houde (1989, 1990) suggested that the larvae of species that inhabit cold environments should be more susceptible to environmental fluctuations than species that inhabit warmer waters as a result of longer development times. Miller et al. (1988) concluded that the survival of species with small offspring should be more variable than the survival of species with larger offspring because of increased vulnerability to starvation and predation with decreasing size. This is consistent with previous hypotheses dealing with the trade-off between offspring size/numbers and potential survival (Smith and Fretwell 1974; Stearns 1980, 1983). However, there has been no comprehensive synthesis of the relationship of development, mortality, and survival rates with temperature and size for all developmental stages of the early life history (i.e. eggs, yolk-sac larvae, feeding larvae) to determine whether Houde's (1989, 1990) and Miller et al.'s (1988) conclusions are consistent throughout the early life history.

The following study presents an empirical summary of development rates, mortality rates, and stage-specific survival of pelagic eggs, yolk-sac larvae, and postlarvae of marine and estuarine fish species in relation to environmental temperature and organism size based on a literature review. The purpose is to determine (1) whether variations in environmental temperature are likely to lead to changes in survival during the early life history and (2) whether decreasing offspring size leads to lower and consequently more variable survival. The analysis is performed on each early life history stage separately because they are physiologically distinct. Choosing to focus only on pelagic stages eliminates possible differences in environmental features that may determine trophic interactions in different habitats (e.g. Sheldon et al. 1972; Schwinghammer 1981; Duarte and Alcaraz 1989). Second, possible effects due to differences in parental care (i.e. energy) that may influence vital rates without being reflected in offspring size or ethological differences that may place offspring into different habitat types (e.g. benthic versus pelagic) are eliminated — all data are for planktonic stages that are dispersed or retained by currents. Third, all field studies used in this analysis are subject to similar constraints of sampling design and gear efficiency that may influence the estimated development, mortality, and survival rates.

The results of this study can provide a preliminary framework for future studies of the early life history of fishes. Deviations from the basic relationships presented herein can be used to assess the association of specific life history adaptations or different environmental factors with variations in survival.

Data Sources and Analysis

All data were gathered from a review of the literature on marine and estuarine fish species. Basic information consisted of egg diameter, length at hatch, and mean length during each study. When information on initial size of eggs and/or larvae was unavailable in specific papers, I referred to reference manuals (Leim and Scott 1966; Russell 1976; Fritzsche 1978; Hardy 1978a, 1978b; Johnson 1978; Jones et al. 1978; Martin and Drewry 1978; Pauly and Pullin 1988; Matarese et al. 1989) to establish the characteristics of that species. When only a size range was available, I used the midpoint between minimum and maximum. Egg diameter was the most widely available index of the initial energy investment by the parent into each offspring. Data on yolk volume which is another and possibly more accurate measure of energy reserves for the larva were often unavailable or poorly estimated.

Daily development and mortality rates, as well as cumulative stage-specific mortality rates, were collated as part of the review. Development times (eq. 2) for eggs and yolk-sac larvae were estimated from laboratory studies in order to minimize error. Development/growth rates of postlarvae were obtained from field studies. Such measurements will be influenced by factors other than temperature (e.g. food availability). However, all data were collected in a similar manner whereas laboratory estimates of larval growth rates may represent rates that are not typical of the field. Daily and cumulative mortality rates of eggs, yolk-sac larvae, and postlarvae were obtained from field studies. Environmental temperature was obtained from the original references. If not specifically stated, I assumed that reported temperatures were those in which the eggs and larvae occurred. When only a temperature range was reported, I used the midpoint between minimum and maximum.

The analysis was performed in two stages. First, I fitted a relationship describing the rates or time periods as a function of temperature. The effect of temperature was determined by fitting an exponential relationship to the combined data for each variable and stage according to

$$(4) \quad y_i = a_i e^{b_i T}$$

where y_i is the response of vital rate i (e.g. development time, rate) to temperature, T (degrees Celsius), at which it was measured and a_i and b_i will be referred to as the scale and temperature parameters, respectively. The model was fitted using ordinary least squares for the transformed equation

$$(5) \quad \ln y_i = \ln a_i + b_i T.$$

Fitting a general relationship with temperature accounts for a common variable in instances where many species spawn within the same temperature range but which differ substantially in the size of their offspring (Ware 1975; Russell 1976; Markle and Frost 1985). Estimating the temperature effect rather than assuming a Q_{10} based on previous studies was done to reduce the possibility of obtaining erroneous results with respect to the effect of body size. Environmental temperature was the strongest correlate of the various rates examined.

The second step consisted of determining whether the residuals from the least squares fit to equation 4 exhibited either an isometric or allometric relationship with the size of the eggs or larvae. The residuals from the regression of the transformed equation (eq. 5) were correlated with the log-transformed measure of organism size using both Pearson product–moment (r) and Kendall's rank (τ) measures of correlation. The nonparametric measure of correlation (τ) assumes a monotonic relationship — a weaker assumption than linearity. A comparison of the parametric (r) and nonparametric (τ) correlation coefficients provides an assessment of the potential influence which outliers might have on the strength of a parametric measure of association such as the product–moment correlation (r) or a regression. If both measures of correlation indicated that there was a significant residual relationship, equation 4 was modified to incorporate a size-dependent term:

$$(6) \quad y_i = a_i e^{b_i T} L^{c_i}$$

where y_i is the response of vital rate i (i.e. time or rate) to temperature, T (degrees Celsius), and size of organism (millimetres). The constants a_i, b_i, and c_i will be referred to as the scale, temperature, and size parameters, respectively.

When there was sufficient taxonomic diversity, I investigated whether groups of similar species exhibited strong departures from the mean in the residuals of the general model which was appropriate to each process and stage (eq. 4 or 6). The four major species groups used in the analysis were based on taxonomic proximity and morphological similarities in early life history characteristics. Anchovies, sardines, and herrings were grouped as clupeoids; cods, haddock, pollocks, and hakes were grouped as gadoids; mackerels, tunas, and sauries were grouped as scombrids; and soles and flounders were grouped as flatfish. For each group, I calculated the mean and standard deviation of the residuals from the general model (eq. 4 or 6). Comparison of the groups was based on ranking the mean residuals. Kendall's tau (τ), a nonparametric measure of association, was used to correlate the rank of different groups in different processes and/or stages. The small number of groups ($n = 4$) prevents any relationship other than a perfect correlation from being significant at the 0.05 level. However, this portion of the study was considered only as exploratory.

Results

Eggs

Increasing temperature (T, degrees Celsius) has a highly significant effect of decreasing egg incubation time (t_h, days) (Fig. 1a). The relationship for 124 species (i.e. the value chosen for each species used in the analysis represented the midpoint or average temperature conditions characteristic of each species) is

$$(7) \quad t_h = 18.7e^{-0.107T}$$

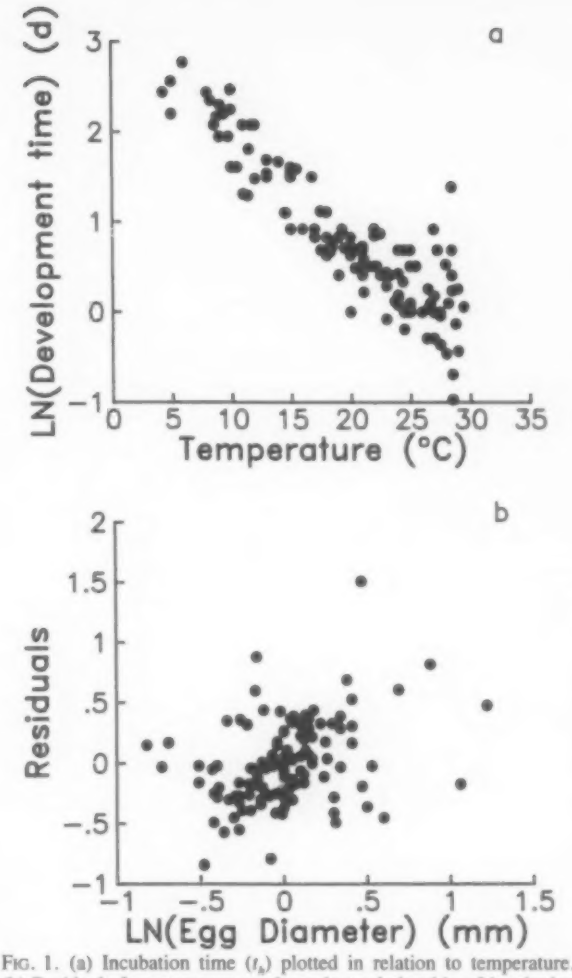

FIG. 1. (a) Incubation time (t_h) plotted in relation to temperature. (b) Residuals from temperature-dependent relationship of incubation times (eq. 7) plotted in relation to the diameter of the egg (Φ).

($r^2 = 0.81$, $p < 0.001$, $n = 124$). The standard error of the temperature parameter is 0.004. Residuals from equation 7 are highly significantly positively correlated with egg size ($r = 0.35$, $p < 0.001$, $\tau = 0.30$, $p < 0.001$, $n = 124$) (Fig. 1b). The general model of time to hatch (t_h, days) in relation to temperature (T, degrees Celsius) and egg diameter (Φ, millimetres) is

$$(8) \quad t_h = 16.1e^{-0.099T}\,\Phi^{0.44}$$

($r^2 = 0.85$, $p < 0.001$, $n = 124$). The standard errors of the temperature and size parameters are 0.004 and 0.099, respectively. Incubation time is shorter as temperature increases. However, larger eggs take longer to develop, suggesting that they should produce larger larvae. Over the range of egg diameters included in this analysis (0.44–3.4 mm) there is a 2.4-fold increase in incubation time.

Estimates of daily mortality rates during the egg stage represent an average from fertilization to hatch. Temperature (T, degrees Celsius) has a highly significant effect on the daily mortality rate of pelagic fish eggs (M_e, per day) (Table 1; Fig. 2a):

TABLE 1. Summary of data on egg diameter (Φ), daily egg mortality rates (M_e), and environmental temperature (T) for the species listed in column 1. Cases with more than one observation represent measurements for different cohorts for which information on temperature was available.

Species	Φ (mm)	M_e (d^{-1})	T (°C)	Source
Engraulis anchoita	1.08	0.60	12.5	Ciechomski and Capezzani 1973
Engraulis capensis	1.07	0.25	18.0	Armstrong et al. 1988
Engraulis japonica	1.08	0.33	19.4	Hiyashi 1966
Engraulis mordax	1.05	0.23	15.0	Smith et al. 1989
	1.05	0.39	15.0	Lo 1985
Engraulis ringens	1.05	1.32	17.0	Smith et al. 1989
	1.05	0.91	17.0	Santander et al. 1983
Etremeus teres	1.33	1.09	22.7	Houde 1977a
Harengula jaguana	1.76	3.64	25.8	Houde 1977c
Melanogrammus aeglefinus	1.45	0.38	5.0	Koslow et al. 1985
Opisthonema oglinum	1.19	2.57	26.4	Houde 1977b
Pleuronectes platessa	1.95	0.039	3.2	Harding et al. 1978
	1.95	0.14	6.6	
	1.95	0.084	7.7	
	1.95	0.063	6.8	
	1.95	0.055	5.6	
	1.95	0.090	4.8	
	1.95	0.074	6.5	
	1.95	0.017	1.5	
	1.95	0.074	6.0	
	1.95	0.12	7.8	
Sardinops caerulea	1.59	0.31	14.0	Smith 1973
Sardinops melanostica	1.50	0.33	15.1	Nakai and Hattori 1962
Sardinops sagax	1.40	2.21	17.0	Smith et al. 1989
Scomber scombrus	1.19	0.40	15.4	Ware and Lambert 1985
	1.19	0.52	17.5	
	1.19	0.41	15.7	
Solea solea	1.30	1.00	10.4	Riley 1974
	1.30	0.60	13.6	
Sprattus sprattus	1.05	0.04	11.5	Alheit et al. 1987
Tautogolabrus adsepsus	0.85	0.67	18.0	Williams and Williams 1973
Trachurus symmetricus	0.95	1.64	15.0	Farris 1961

(9) $M_e = 0.030e^{0.187T}$

($r^2 = 0.72$, $p < 0.001$, $n = 32$). The standard error of the temperature parameter is 0.020. Residuals from equation 9 were not significantly correlated with egg diameter ($r = -0.08$, $p > 0.1$, $\tau = -0.18$, $p > 0.1$, $n = 32$) (Fig. 2b). Daily mortality rates of pelagic fish eggs increase with increasing temperature but are independent of size.

There is evidence that some of the residual variance in the general models of time to hatch (eq. 8) and daily mortality rates (eq. 9) of eggs shows an association with certain species groups. Table 2 lists the means and standard deviations of residual development times and mortality rates for the different groups. The pattern indicates that groups that tend to have short development times, beyond that described by the general temperature- and size-dependent model (eq. 8), have lower average daily mortality rates ($\tau = 0.67$, $p = 0.17$, $n = 4$). It is possible that species/taxonomic groups that have longer embryonic periods present a more visible target for predators which could result in higher average mortality rates. This association does not appear to be due to size differences (eq. 9).

Because the temperature parameter for the daily mortality rate of eggs is greater than the temperature parameter for development times, incorporating the general relationshps for development time (eq. 8) and daily mortality rates (eq. 9) into equation 3 would predict that cumulative egg mortality should increase with increasing temperature. Furthermore, because development times increase with increasing size but daily mortality rates of egg are independent of size, the prediction would be that cumulative mortality rates should increase with increasing size. Analysis of cumulative mortality rates (C_e) shows a highly significant positive effect of temperature (Fig. 3a):

(10) $C_e = 0.65e^{0.066T}$

($r^2 = 0.31$, $p < 0.01$, $n = 32$). The standard error of the temperature parameter is 0.019. Residuals from equation 10 were not significantly correlated with egg diameter ($r = -0.04$, $p > 0.1$, $\tau = -0.09$, $p > 0.2$, $n = 32$) (Fig. 3b), in contrast with the predictions. The temperature parameter of equation 10 is not significantly different ($t = 0.79$, $p > 0.1$, $n = 32$) from the value that would have been predicted based on the results of equations 8 and 9.

Yolk-Sac Larvae

During incubation, the energy placed into each egg will be divided into development of the embryo and energy reserves for the larva after hatching, with some metabolic losses due to respiration. The size of the larva at hatch (i.e. data for this analysis represents a summary for species found in the mid-Atlantic Bight (Fritzsche 1978; Hardy 1978a, 1978b; Johnson 1978; Jones et al. 1978; Martin and Drewry 1978), the North Sea (Russell 1976), waters off Japan (Shirota 1970), and the

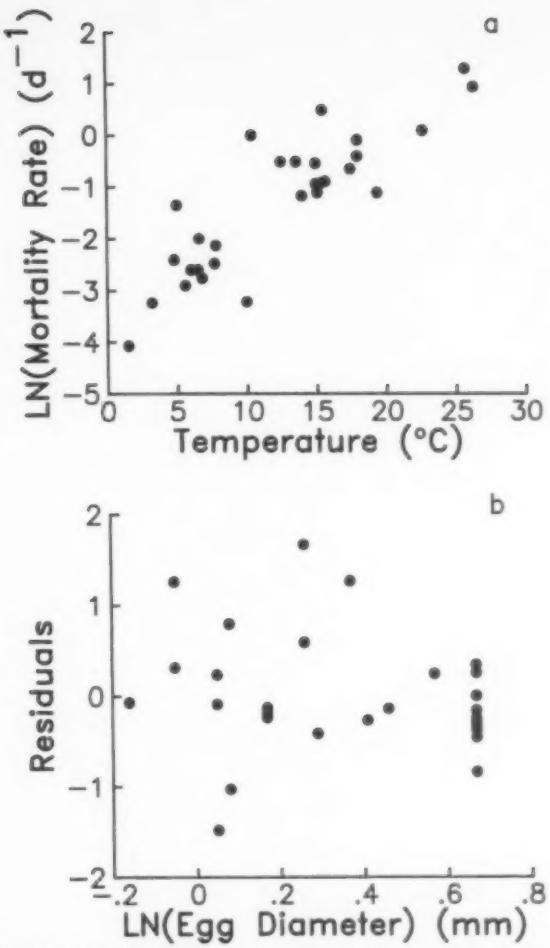

FIG. 2. (a) Daily instantaneous mortality rates of pelagic fish eggs (M_e), estimated from field surveys, in relation to environmental temperature. (b) Residuals from temperature-dependent relationship of mortality rates (eq. 9) plotted in relation to egg diameter (Φ).

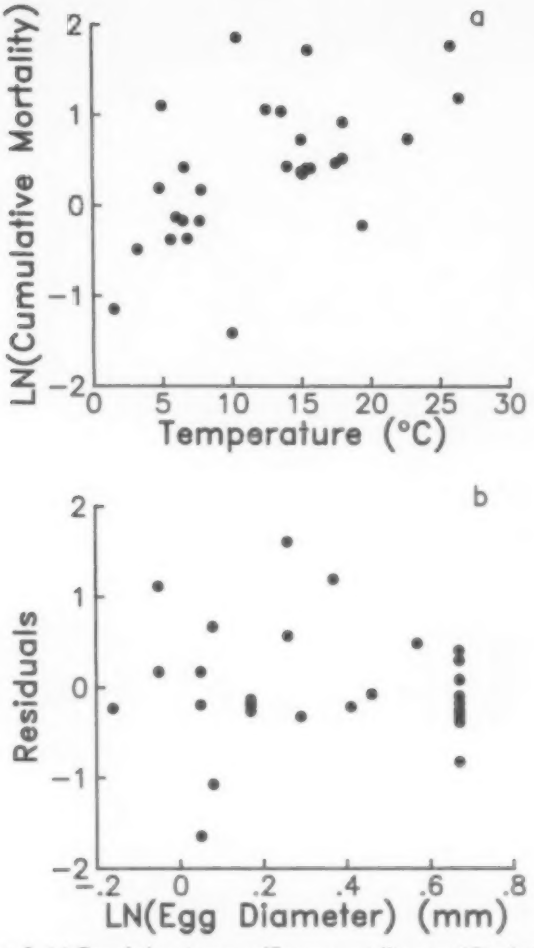

FIG. 3. (a) Cumulative stage-specific egg mortality rates (C_e) plotted in relation to environmental temperature. (b) Residuals from temperature-dependent relationship of cumulative stage-specific egg mortality rates (eq. 10) plotted in relation to egg diameter (Φ).

TABLE 2. Mean and standard deviation of the log-transformed residuals (R) for equations 8 and 9, which describe the time to hatch and the daily mortality rates of eggs in relation to temperature and, in the case of the former, size for different taxonomic groups of pelagic fish eggs. The standard deviations (SD) of all log-transformed residuals of equations 8 and 9 are 0.32 and 0.67, respectively.

Taxonomic group	Development times			Daily mortality rates		
	$\bar{R}$	SD	n	$\bar{R}$	SD	n
Clupeoids	−0.097	0.35	9	−0.24	0.54	10
Gadoids	0.20	0.22	11	1.27	—	1
Scombrids	0.041	0.41	12	0.21	0.63	5
Flatfish	0.085	0.21	12	0.01	0.65	12

Northeast Pacific (Matarese et al. 1989)) increases with increasing egg size (Fig. 4):

$$(11)\quad L_0 = 2.89\,\Phi^{0.89}$$

($r^2 = 0.62$, $p < 0.001$, $n = 187$) where L_0 is the length at hatch (millimetres) and Φ is egg diameter (millimetres) and the standard error of the exponent is 0.061. The exponent is not significantly different from 1 ($t = 1.80$, $p > 0.05$, $n = 187$). This result is consistent with findings by Duarte and Alcaraz (1989) which also showed that hatchling length is an isometric function of egg diameter. If we assume that the mass of eggs and larvae, per unit length, are approximately equal ($W \propto l^3$), this implies that as egg diameter increases, the proportion of mass placed into the larva does not change with increasing egg size. By difference, this implies that the proportion of egg mass placed into energy reserves for the hatchling will also be an isometric function of egg mass. Because larval fish respiration rates are an isometric function of their size (Giguère et al. 1989), the duration of larval stages dependent on endogenous energy reserves (e.g. time to yolk absorption and the point of no return) should be independent of initial size when comparing species.

Time to yolk absorption (t_a, days) defines the period during which the larva can rely on endogenous energy reserves and thus determines the time available for larvae to find suitable food resources. Analysis of data listed in Table 3 reveals that increasing temperature (T, degrees Celsius) reduced the duration of the yolk-sac stage (Fig. 5a). The relationship is

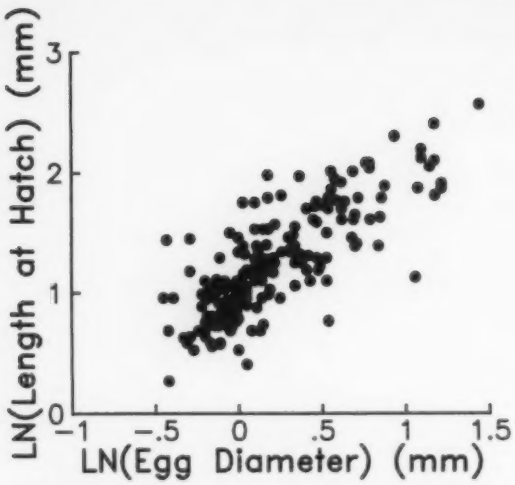

FIG. 4. Length at hatch (L_0) plotted in relation to egg diameter (Φ).

(12) $t_a = 19.9e^{-0.071T}$

($r^2 = 0.49$, $p < 0.001$, $n = 41$). The standard error of the temperature parameter is 0.011. The residuals from equation 12 are not significantly correlated with length at hatch ($r = -0.05$, $p > 0.20$, $\tau = -0.03$, $p > 0.20$, $n = 41$) (Fig. 5b). Comparatively, increasing either egg or larval size will not lead to a relatively longer period until yolk absorption.

Another indication of the ability of larvae to withstand food deprivation is the point of no return which measures the period of food deprivation, from hatch, after which starvation is irreversible. There is a very significant negative relationship between the period until the point of no return (t_{pnr}, days) and temperature (T, degrees Celsius) (Table 3; Fig. 6a):

(13) $t_{pnr} = 42.1e^{-0.081T}$

($r^2 = 0.69$, $p < 0.001$, $n = 29$). The standard error of the temperature parameter is 0.01. Residuals from equation 13 are not significantly correlated with length at hatch ($r = 0.26$, $p > 0.1$, $\tau = 0.13$, $p > 0.2$, $n = 25$) (Fig. 6b). The difference in scale and temperature parameters of equations 12 and 13 indicates that (1) the time until the point of no return is substantially longer than the time required for yolk absorption and (2) the point of no return appears to be more sensitive to increases in temperature than the time until yolk absorption. Consequently, species that inhabit cold waters will have a relatively longer time period between the stage when they can no longer rely on endogenous energy reserves and the stage at which failure to successfully feed becomes fatal. The lack of any significant effect of initial size on time to yolk absorption or to the point of no return is consistent with the prediction based on the relationship between egg and larval sizes (eq. 11).

When grouped by taxonomic categories (clupeoids, gadoids scombrids, and flatfishes), there is an indication that the average residuals in time to irreversible starvation (Table 4) and the residuals in egg development times (Table 2) are not strongly correlated ($\tau = 0.33$, $p = 0.5$, $n = 4$). This suggests that the longer development times during the egg stage do not influence the amount of energy reserves for the hatchlings.

There are few estimates of daily mortality rates specifically for yolk-sac larvae. In addition to Dalhberg's (1979) summary, average mortality rates from Hewitt et al. (1985), Fortier and Leggett (1985), and Taggart and Leggett (1987) were the only studies to estimate mortality specifically during the yolk-sac stage (Table 5). There is no significant relationship of mortality with either temperature ($r = -0.19$, $p > 0.2$, $\tau = -0.15$, $p > 0.2$, $n = 13$) (Fig. 7a) or length ($r = -0.36$, $p > 0.15$, $\tau = -0.27$, $p > 0.15$, $n = 13$) (Fig. 7b). The average daily mortality rate of yolk-sac larvae (M_{ys}, per day) is 0.33 (SE = ± 0.061).

As a result of the strong effect of temperature on development times (eq. 12; Fig. 5a), cumulative mortality rates during the yolk-sac stage (C_{ys}) show a significant relationship with temperature (T, degrees Celsius) (Fig. 8a):

(14) $C_{ys} = 3.99e^{-0.061T}$

($r^2 = 0.30$, $p = 0.05$, $n = 13$). The standard error of the temperature parameter is 0.04. The residuals from this relationship are not significantly correlated with length at hatch ($r = -0.32$, $p > 0.05$, $n = 13$) (Fig. 8b).

Postlarvae

Growth rates of postlarvae (G, millimetres per day) are very significantly positively correlated with temperature (T, degrees Celsius) (Table 6; Fig. 9a):

(15) $G = 0.12e^{0.063T}$

($r^2 = 0.35$, $p < 0.001$, $n = 53$). Residuals from this relationship are not significantly correlated with the initial length of the larvae (L_0) ($r = -0.05$, $p > 0.1$, $\tau = -0.06$, $p > 0.2$, $n = 53$), which implies that the length at hatch may not be a good indicator of the larva's foraging ability. However, residuals are very significantly correlated with the average length of the larva during the period of measurement (L) ($r = 0.40$, $p < 0.01$, $\tau = 0.27$, $p < 0.01$, $n = 53$) (Fig. 9b) such that

(16) $G = 0.031e^{0.073T}L^{0.54}$

($r^2 = 0.45$, $p < 0.001$, $n = 53$). The standard errors of the temperature and size parameters are 0.012 and 0.018, respectively. The standard error terms are large because of the unknown influence of food availability. As with the egg and yolk-sac larvae, development rates are more rapid in warm waters. The association of increased growth rates with increasing size indicates that better foraging ability is associated with advancing development. The larger/older larva not only has higher capture success (Hunter and Thomas 1974) but can also search larger volumes (Miller et al. 1988). However, the initial length does not appear to result in a significantly greater foraging ability.

Daily mortality rates of postlarvae (M_l, per day) are very significantly positively correlated with temperature (T, degrees Celsius) (Table 6; Fig. 10a):

(17) $M_l = 0.044e^{0.077T}$

($r^2 = 0.48$, $p < 0.001$, $n = 32$). The residuals from equation 17 are significantly negatively correlated with the length at hatch (L_0) ($r = -0.44$, $p < 0.05$, $\tau = -0.32$, $p < 0.05$, $n = 32$) as well as with the average length over the study period (L) ($r = -0.46$, $p < 0.05$, $\tau = -0.33$, $p < 0.05$, $n = 32$) (Fig. 10b) such that the general relationship is

(18) $M_l = 0.25e^{0.067T}L^{-0.68}$

TABLE 3. Egg diameter (Φ), length at hatch (L_0), time to yolk absorption (t_a), point of no return (t_{pnr}), and the experimental temperature (T) for various species of marine and estuarine pelagic yolk-sac larval fish. All experiments were conducted in the laboratory. Sources of information are reported in the right-hand column. The abbreviation "gen.cit." refers to information previously summarized by Fritzsche (1978), Hardy (1978a, 1978b), Johnson (1978), Jones et al. (1978), and Martin and Drewry (1978).

Species	Φ (mm)	L_0 (mm)	t_a (d)	t_{pnr} (d)	T (°C)	Source
Achirus lineatus	1.90	2.7	3.5	4.0	24.0	Houde 1974
Alosa aestivalis	1.00	4.3	2.5	—	22.0	gen.cit.
Alosa pseudoharengus	1.04	3.8	3.5	5.5	12.0	Rice 1985
Alosa sapidissima	3.15	7.8	7.0	—	12.0	gen.cit.
Anchoa hepsetus	1.13	3.8	1.0	—	20.0	gen.cit.
Anchoa mitchilli	0.82	2.3	2.5	3.4	28.0	Houde 1974
Archosargus rhomboidalis	0.85	2.0	3.1	4.0	26.0	Houde 1974
Aspidoparia morax	—	4.1	—	14.0	14.0	Malhotra and Munshi 1985
Auxis sp.	0.85	2.3	6.0	—	27.0	gen.cit.
Caranx mate	0.72	—	5.0	7.4	25.0	Struhsaker et al. 1973
Centropristis striata	0.95	2.0	3.5	—	16.0	gen.cit.
Chanos chanos	1.20	3.4	3.0	5.0	29.0	Liao et al. 1979
Clupea harengus	1.20	6.8	26.0	30.0	8.0	Blaxter and Hempel 1963; Blaxter and Ehrlich 1974
Clupea pallasi	1.35	7.5	21.0	28.0	8.0	McGurk 1984
Dicentrarchus labrax	1.18	—	15.7	31.0	15.0	Barnabé et al. 1976
Dorosoma petenese	0.75	4.2	3.0	—	27.0	gen.cit.
Engraulis mordax	1.05	2.8	6.0	7.5	16.5	Lasker et al. 1970
Engraulis ringens	1.07	2.9	5.3	6.8	18.0	Mendiola and Gomez 1981
Epinephelus tauvina	0.84	2.3	5.2	7.2	28.0	Hussain and Higuki 1980
Eurythunnus alletteratus	0.89	3.0	2.0	—	25.0	gen.cit.
Gadus morhua	1.53	3.0	18.0	23.0	7.0	Ellertsen et al. 1980
Katsuwonus pelamis	0.99	2.7	2.0	—	26.0	gen.cit.
Leiostomus xanthurus	1.60	—	—	20.0	8.0	Powell and Chester 1985
Leuresthes tenuis	—	9.0	—	18.0	16.0	May 1971
Melanogrammus aeglefinus	1.25	3.5	17.5	22.5	7.0	Laurence and Rogers 1976
Melanogrammus aeglefinus	1.46	3.5	10.0	—	5.0	gen.cit.
Merluccius productus	1.13	3.5	6.7	8.0	12.0	Bailey 1982
Microgadus tomcod	1.57	5.0	5.0	—	4.4	gen.cit.
Morone saxatilis	2.90	3.1	3.0	—	24.0	gen.cit.
Mugil cephalus	1.27	2.6	5.0	9.0	22.5	Nash et al. 1974
Pleuronectes platessa	1.95	7.2	20.1	28.0	10.0	Ryland and Nichols 1975
Pollachius virens	1.15	3.5	5.0	—	4.4	gen.cit.
Pseudopleuronectes americanus	0.82	2.9	27.0	33.0	8.0	Beyer and Laurence 1980
Sardina pilchardus	1.60	—	5.8	1.0	17.0	Blaxter 1969
Scomber japonicus	1.05	3.2	5.3	6.8	19.0	Hunter and Kimbrell 1980
Scomber scombrus	1.20	3.5	5.7	—	13.1	gen.cit.
Scomberomorus maculatus	1.00	2.6	4.0	—	22.0	gen.cit.
Scophthalmus maeoticus	1.10	2.9	9.4	12.5	16.0	Spectorova et al. 1974
Siganus fuscensens	0.60	2.6	4.2	5.2	25.0	Lam 1974
Siganus oramin	0.44	1.6	3.4	4.2	27.0	Westernhagen and Rosenthal 1975
Siganus rivulatus	0.50	1.8	5.4	6.4	27.0	Popper et al. 1973
Solea solea	1.25	3.2	15.0	21.0	11.0	Riley 1974
Stizostedion vitreum	1.85	6.8	9.5	—	13.9	gen.cit.
Thunnus albacores	0.98	2.7	3.0	—	27.0	gen.cit.
Trachurus symmetricus	0.95	2.5	8.0	10.5	15.0	Theilacker 1978
Trinectes maculatus	0.84	1.8	2.0	—	23.0	gen.cit.
Urophycis chuss	0.72	1.9	1.3	—	21.0	gen.cit.

($r^2 = 0.59$, $p < 0.001$, $n = 32$) where L is the mean length of the larvae over the study period and T is temperature (degrees Celsius). The standard errors of the temperature and size parameters are 0.013 and 0.23, respectively.

Instantaneous daily growth and mortality rates can be linked both from an evolutionary perspective (Ware 1975) as well as from a mechanistic one (L. Giguère, Institut Maurice-Lamontagne, Mont-Joli (Qué.), pers. comm.; R. Dunbrack, Biology Department, Memorial University of Newfoundland, St. John's, Nfld., pers. comm.). Ware (1975) argued that in order for a cohort to increase in weight over time, mortality rates must be less than growth rates. It follows that in order to grow rapidly, organisms must increase activity levels to encounter and ingest a greater number of prey items. Increased activity on the part of the larvae will lead to increased encounter rates with both prey and predators (Gerritsen and Strickler 1977) and thus lead to higher daily mortality rates. There is a strong relationship between average daily mortality rates and average daily growth rates (Fig. 11a):

$$(19) \quad M_i = 0.32G^{0.62}$$

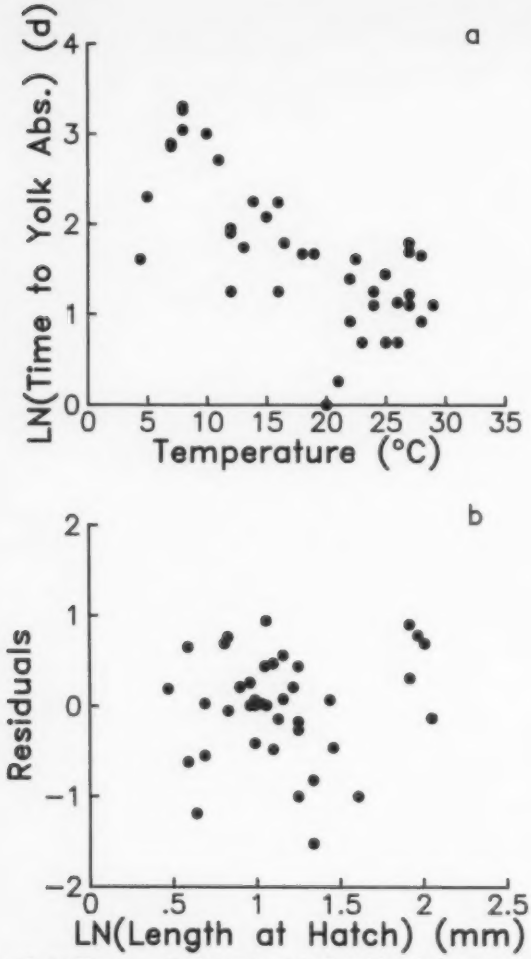

FIG. 5. (a) Time to yolk absorption (t_a) plotted in relation to temperature. (b) Residuals from temperature-dependent relationship of time to yolk absorption (eq. 12) plotted in relation to length at hatch (L_0).

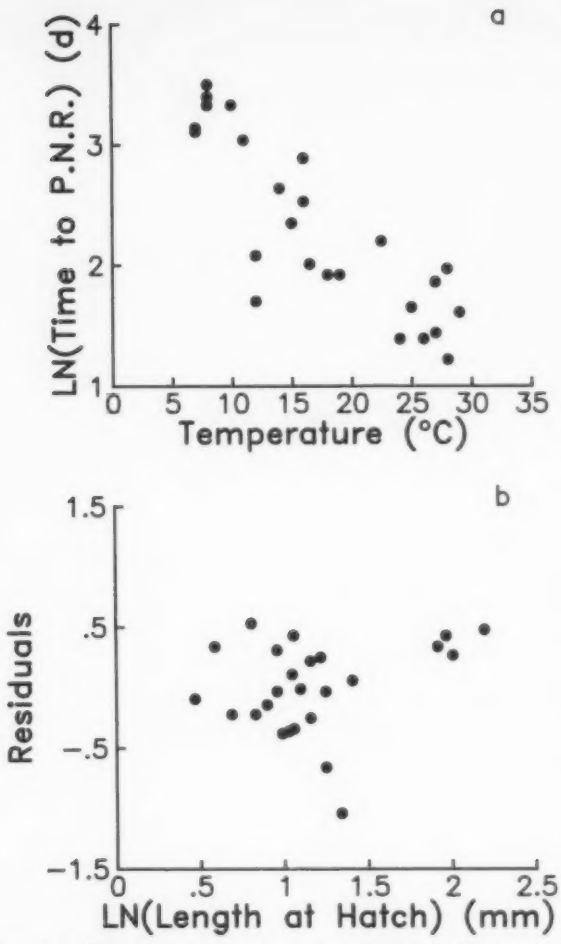

FIG. 6. (a) Time to point of no return (t_{pnr}) plotted in relation to temperature. (b) Residuals from temperature-dependent relationship of time to point of no return (eq. 13) plotted in relation to length at hatch (L_0).

($r^2 = 0.38$, $p < 0.001$, $n = 32$). Species that grow rapidly have high mortality rates. Residuals from this relationship are significantly correlated with the average size ($r = -0.77$, $p < 0.01$, $\tau = -0.58$, $p < 0.01$, $n = 32$) (Fig. 11b) such that

$$M_I = 5.17G^{0.74}L^{-1.17} \quad (20)$$

($r^2 = 0.76$, $p < 0.001$, $n = 32$). Mortality rates decrease with increasing size. The standard errors of the growth and size parameters are 0.09 and 0.17, respectively. It is clear from these results that species which grow more rapidly also suffer higher mortality rates but that mortality rates decrease with increasing size.

Beyer (1989) suggested that the ratio of daily mortality and development rates ($C_I = M_I/G$) provides a measure of the physiological rate of mortality which may also be taken as a measure of the stage-specific or length-dependent (Ebert 1973) mortality rates. Analysis of the data (Table 6) reveals that stage-specific mortality rates of postlarval fish (C_I) are not significantly correlated with temperature ($r = -0.03$, $p < 0.2$, $\tau = 0.01$, $p > 0.2$, $n = 32$) (Fig. 12a). This is the result of the effects of temperature on daily growth and mortality rates cancelling each other (cf. eq. 1) because the temperature parameters of the general models (eq. 16 and 18) are not significantly different ($t = 0.4$, $p > 0.2$, $n_1 = 32$, $n_2 = 53$). Postlarval stage-specific mortality rates are only marginally correlated with initial length ($r = -0.30$, $0.1 > p > 0.05$, $\tau = -0.30$, $0.1 > p > 0.05$, $n = 85$) although they show a very strong relationship with the average size of the larvae over the study period (Fig. 12b):

TABLE 4. Mean and standard deviation of the log-transformed residuals (R) for equation 12, describing the time to yolk absorption in relation to temperature, for different taxonomic groups of pelagic fish eggs. The standard deviation (SD) of all log-transformed residuals of equation 12 is 0.32.

Taxonomic group	Development times		
	$\bar{R}$	SD	n
Clupeoids	−0.18	0.19	14
Gadoids	−0.16	0.42	3
Scombrids	−0.24	0.06	2
Flatfish	0.07	0.48	3

TABLE 5. Environmental temperature (T), length at hatch (L_0), and daily mortality rates (M_{ys}) of yolk-sac larvae as summarized by Dalhberg (1979) with some additional references (see text).

Species	L_0 (mm)	M_{ys} (d^{-1})	T (°C)	Source
Clupea harengus	6.5	0.123	6.0	Dragesund and Nakken 1973
Engraulis ponticus	3.1	0.641	29.0	Dekhnik 1963
Etremeus teres	4.3	0.173	13.0	Houde 1977a
Harengula jaguana	4.0	0.298	14.0	Houde 1977c
Mallotus villosus	5.0	0.620	8.0	Fortier and Leggett 1985
Mallotus villosus	5.5	0.560	6.0	Taggart and Leggett 1987
Opisthonema oglinum	3.0	0.186	19.0	Houde 1977b
Pleuronectes platessa	5.9	0.043	11.6	Ryland 1966
Sardinops caerulea	3.8	0.101	22.0	Ahlstrom 1954
Sardinops melanostica	5.0	0.274	22.0	Nakai and Hattori 1962
Scomber scombrus	3.5	0.200	13.0	Sette 1943
Trachurus symmetricus	3.3	0.511	26.0	Farris 1961
Trachurus trachurus	3.2	0.650	16.0	Hewitt et al. 1985

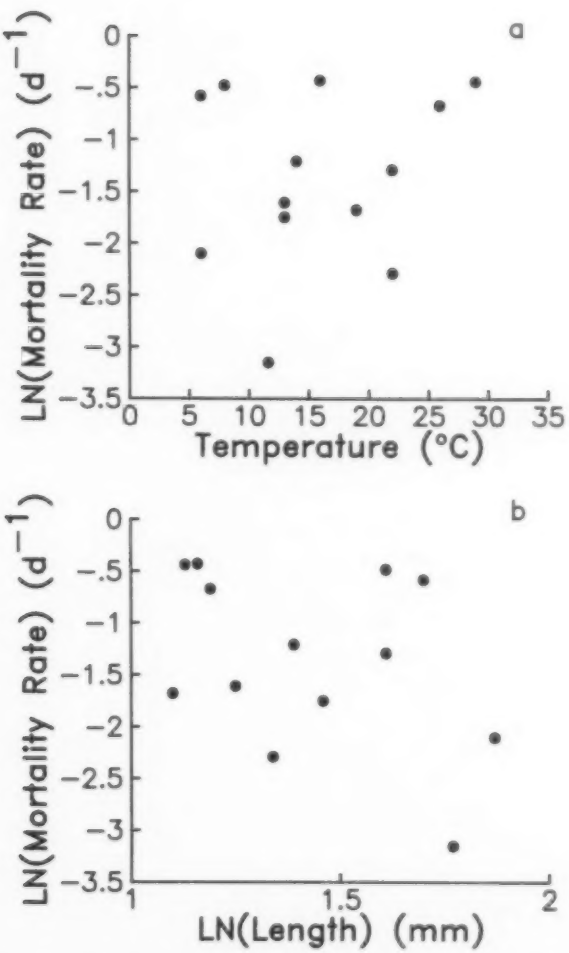

FIG. 7. (a) Daily instantaneous mortality rates of yolk-sac larvae (M_{ys}) plotted in relation to environmental temperature. (b) Daily instantaneous mortality rates of yolk-sac larvae plotted in relation to length at hatch (L_0).

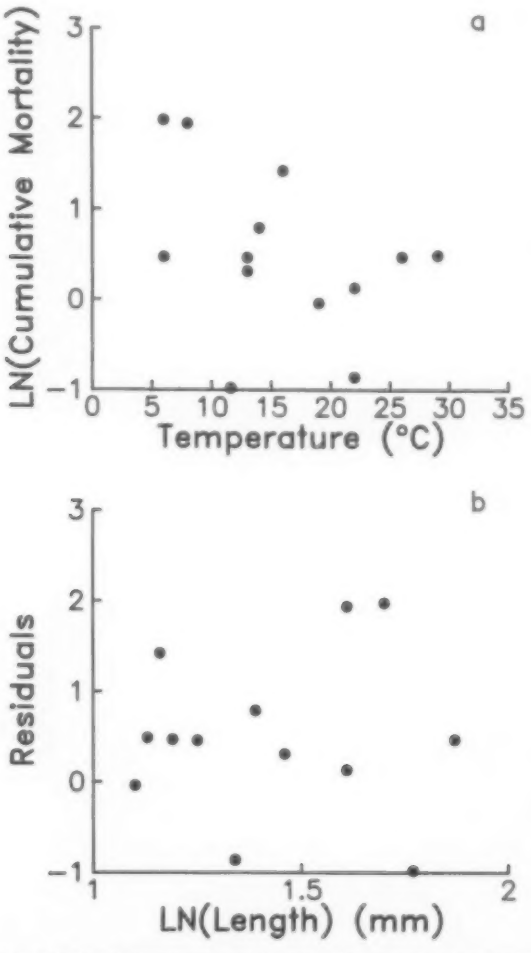

FIG. 8. (a) Cumulative stage-specific mortality rates of yolk-sac larvae (C_{ys}) plotted in relation to environmental temperature. (b) Residuals from temperature-dependent relationship of cumulative stage-specific mortality rates of yolk-sac larvae (eq. 14) plotted in relation to length at hatch (L_0).

TABLE 6. Minimum (L_o) and maximum (L_f) length of the size range of larvae for which field growth (G_f) and mortality (M_f) rates were estimated and mean environmental temperature (T). Species with more than one observation represent measurements for different cohorts for which temperature data were available. Data for northern anchovy (*Engraulis mordax*) (Zweifel and Smith 1981) represent the average for 79 quarterly estimates assuming a mean growth rate of 0.4 mm/d. An asterisk next to the reference indicates that measurements were taken in mesocosms.

Species	L_o (mm)	L_f (mm)	G_f (mm/d)	M_f (d^{-1})	T (°C)	Source
Anchoa mitchilli	3.4	12.5	0.56	0.32	29.0	Leak and Houde 1987
	3.4	12.5	0.43	0.45	24.4	
	3.4	12.5	0.48	0.30	28.1	
	3.4	12.5	0.48	0.42	30.7	
Archosargus rhomboidalis	4.0	8.0	0.25	0.18	24.0	Chavance et al. 1984
Cololabis saira	7.5	57.5	1.16	0.07	14.5	Watanabe and Lo 1989
Clupea harengus	7.2	18.0	0.18	—	8.0	Munk et al. 1986
Clupea harengus	5.7	17.0	0.28	—	8.0	Lough et al. 1982
Clupea harengus	6.0	30.0	0.20	—	10.0	Graham and Townsend 1985
Clupea harengus	6.0	35.0	0.29	—	10.0	Townsend and Graham 1981
Clupea harengus	7.0	16.0	0.14	—	7.5	Gamble et al. 1985*
	7.0	34.0	0.36	—	11.0	
Clupea harengus	6.5	30.4	0.19	0.04	8.0	Lough et al. 1981
Clupea harengus	6.7	11.4	0.18	0.06	7.0	Henderson et al. 1984
Clupea pallasi	7.7	30.0	0.39	0.09	11.0	McGurk 1987
	7.7	18.0	0.40	0.09	12.0	
	7.7	21.4	0.36	0.31	11.0	
	7.7	29.0	0.41	0.06	10.0	
Engraulis mordax	2.8	20.0	0.37	—	15.0	Methot and Kramer 1979
Engraulis mordax	3.0	15.0	0.40	0.22	15.5	Zweifel and Smith 1981
Etrumeus teres	4.5	16.0	0.50	0.13	24.0	Houde 1977a
	4.5	16.0	0.50	0.13	24.0	
Gadus morhua	3.0	6.5	0.12	—	8.0	Gamble and Houde 1984*
Gadus morhua	4.0	22.0	0.18	—	6.0	Bolz and Lough 1983
Leiostomus xanthurus	1.6	16.0	0.20	—	19.3	Warlen and Chester 1985
Mallotus villosus	5.0	33.0	0.25	—	8.0	Jacquaz et al. 1977
Melanogrammus aeglefinus	3.1	12.7	0.21	—	7.0	Bolz and Lough 1983
Merluccius productus	3.0	6.0	0.16	—	12.0	Bailey 1982
Opisthonema oglinum	6.5	19.0	0.80	0.21	26.0	Houde 1977b
	6.5	19.0	0.80	0.26	26.0	
Parophrys vetulus	2.9	12.0	0.33	—	10.0	Laroche et al. 1982
Pleuronectes platessa	7.0	11.5	0.04	0.06	6.0	Harding and Talbot 1973
Pseudopleuronectes americanus	6.6	8.6	0.14	—	14.5	Laurence et al. 1978*
Pseudopleuronectes americanus	3.5	6.0	0.16	0.12	14.5	Pearcy 1962
Sardina pilcharus	3.7	18.0	0.46	—	10.0	Ré 1983
Sardinella aurita	3.0	12.0	0.96	0.46	23.0	Conand and Fagetti 1971; Conand 1977
Sardinops melanostica	5.0	15.0	0.23	0.10	15.1	Nakai and Hattori 1962
Sardinops sagax	3.0	16.0	0.80	—	18.5	Butler and Mendiola 1985
Scomber japonicus	2.8	15.0	0.64	0.32	18.0	Watanabe 1970
Scomber scombrus	3.2	15.0	0.73	—	16.0	Lambert and Ware 1984
Scomber scombrus	3.2	8.0	0.57	0.38	15.4	Ware and Lambert 1985
	3.2	8.0	0.59	0.53	15.7	
	3.2	8.0	0.69	0.71	17.3	
Scopthalmus maximus	3.0	6.5	0.34	—	20.5	Rosenberg and Haugen 1982*
Sebastes sp.	6.4	17.9	0.16	0.04	7.0	Anderson 1984
	6.4	9.0	0.04	0.04	6.8	
	9.0	11.3	0.07	0.06	8.8	
	11.3	21.4	0.26	0.13	11.5	
Theragra chalcogramma	3.8	13.0	0.24	—	6.0	Kendall et al. 1987; Incze et al. 1989
Theragra chalcogramma	4.0	22.0	0.35	—	7.0	Walline 1985
Trachurus symmetricus	3.3	5.1	0.16	0.28	15.0	Hewitt et al. 1985
Trachurus symmetricus	3.3	12.0	0.22	0.18	15.0	Hewitt 1981
Urophycis tenuis	3.2	12.0	0.70	—	16.0	Lambert and Ware 1984

(21) $C_l = 8.5\bar{L}^{-1.27}$

($r^2 = 0.59$, $p < 0.001$, $n = 32$). The standard error of the size parameter is 0.18. The implication is that as larval fish increase in size, the mortality per unit growth decreases.

There is an indication of a notable taxonomic effect, beyond that accounted for by environmental temperature and organism size, in the residual daily growth (eq. 16) and mortality (eq. 18) rates. As with the general relationship, species groups that grow rapidly tend to have high mortality rates ($\tau = 0.67$, $p = 0.17$, $n = 5$) (Table 7). Scombrids and gadoids grow more rapidly than clupeoids, flatfishes, and rockfish (*Sebastes* spp.). When ranked relative to one another, taxonomic groups that have high postlarval growth rates are groups that tend to have short periods until the point of no return ($\tau = -0.67$, $p = 0.17$, $n = 4$)

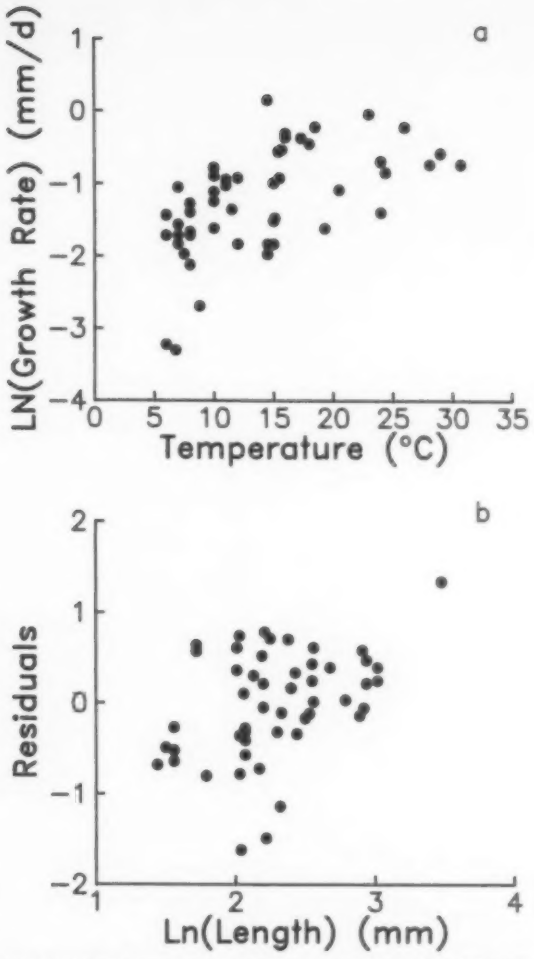

FIG. 9. (a) Daily average growth rates of postlarval fish (G) plotted in relation to environmental temperature. (b) Residuals from temperature-dependent relationship of growth rates of postlarval fish (eq. 15) plotted in relation to mean length during the study period (L).

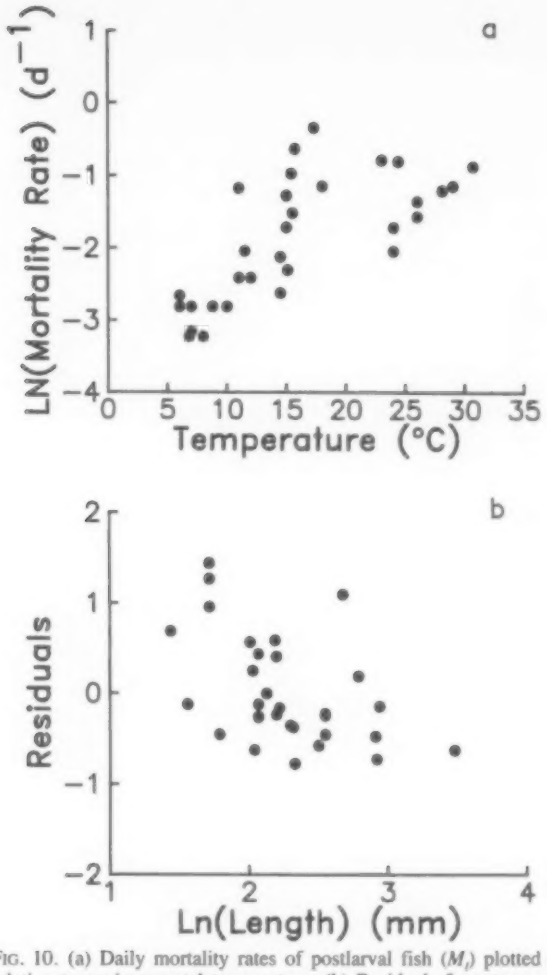

FIG. 10. (a) Daily mortality rates of postlarval fish (M_j) plotted in relation to environmental temperature. (b) Residuals from temperature-dependent relationship of daily mortality rates of postlarval fish (eq. 17) plotted in relation to mean length during the study period (L).

(Table 4). *Sebastes* spp. are excluded from this correlation because of differences in developmental patterns (Penney 1987). Species groups that are intrinsically fast growers or effective feeders place relatively less energy into reserves for the hatchlings in contrast with species groups that appear to have intrinsically slower growth rates.

Discussion

Variations in temperature do not appear to have any significant net effect on early life history survival (Table 8). Although temperature has a significant influence on stage-specific cumulative mortality of fish eggs and yolk-sac larvae, its effect on one stage is equal and opposite to its effects in the other. Initial size does not appear to significantly influence survival in either egg, yolk-sac, or postlarval stages, although the average size per se does influence the estimated survival of postlarval fishes (Table 8). These results are in contrast with previous conclusions that the early life history survival should be more variable for species that spawn in cold environments (Houde 1989, 1990) or for species that produce small eggs relative to other species that produce larger eggs or larvae (Mille et al. 1988). The discrepancy between the conclusions of this and other studies is likely to be due to studying the elements of the survival process separately rather than studying development and mortality rates simultaneously.

Temperature had a significant influence on almost all the estimated daily development and mortality rates. An increase in temperature leads to more rapid development during all the early life history stages. Furthermore, the development rate of eggs is more sensitive to variations in temperature than is the development rate of larvae. Similar conclusions can be applied to daily mortality rates. Temperature increases lead to increases in daily mortality rates during both egg and postlarval stages although there appears to be no significant influence during the yolk-sac stage. As with developmental rates, daily mortality rates of postlarval fishes are less sensitive to temperature fluctuations than daily mortality rates of eggs. When relationships for daily development and mortality rates for each stage are combined using equation 1, it is possible to predict that there is an influence of temperature on cumulative mortality during

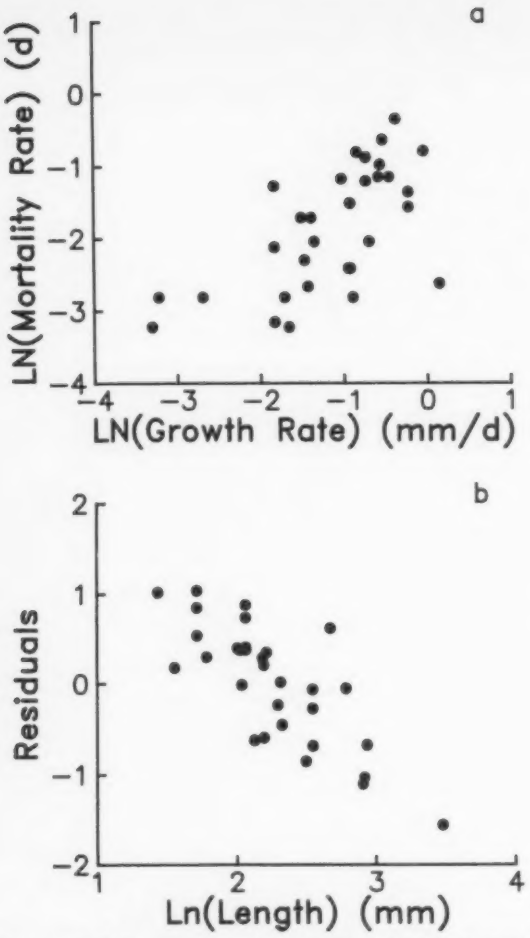

FIG. 11. (a) Daily mortality rates of postlarval fish (M_i) plotted in relation to average daily growth rates (G). (b) Residuals from growth-dependent relationship of daily mortality rates of postlarval fish (eq. 19) plotted in relation to mean length during the study period (L).

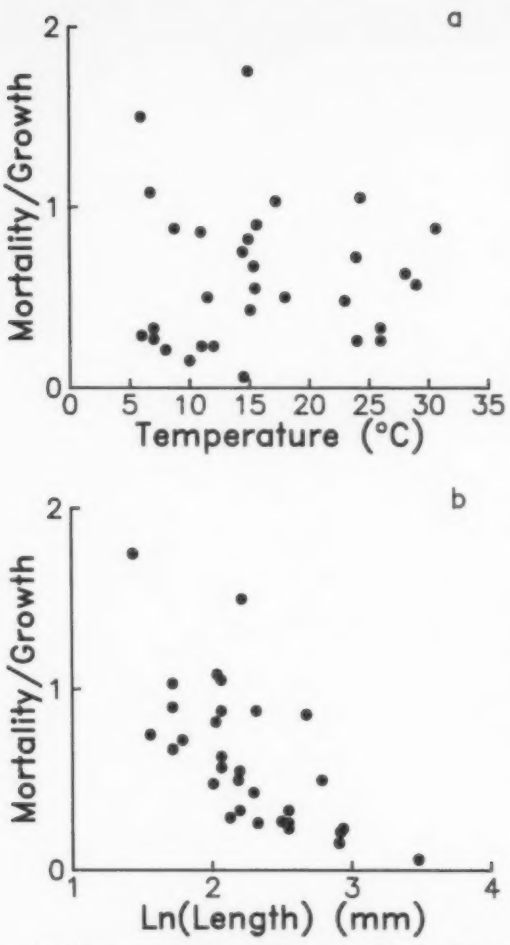

FIG. 12. (a) Daily stage specific mortality rates (C_i) plotted in relation to environmental temperature. (b) Daily stage-specific mortality rates (C_i) plotted in relation to mean length during the study period (L).

the egg and yolk-sac stages. However, the effect on each stage is of the same magnitude (i.e. the temperature parameters of equations 10 and 14 are not significantly different in absolute value) but opposite. The net result is that temperature fluctuations have little or no impact on cumulative mortality from spawning to yolk absorption. Because large-scale temperature anomalies persist for several months in marine systems (Myers et al. 1988), a warm period that may lead to higher losses during the egg stage, for example, should also lead to an approximately equal reduction in losses during the yolk-sac stage. Because there is no net effect of temperature on postlarval stage-specific mortality rates (eq. 21), I suggest that variations in temperature are likely to have minor effects on early life history survival, assuming that increased temperature does not have other potentially detrimental or beneficial effects on other elements of the food web. This is in contrast with Houde's (1989, 1990) suggestion that there is a strong latitudinal pattern in sensitivity to temperature fluctuations.

Compensation for the effect of variations in temperature, either in different stages (egg versus yolk-sac larvae) or in different physiological processes (postlarval growth and mortality), may represent an adaptive response to minimize the effect of environmental unpredictability within the normal range of conditions for each species. For example, a typical range of temperature anomalies in average monthly sea surface temperature (degrees Celsius) spans approximately 4° (S. A. Akenhead, Northwest Atlantic Fisheries Center, St. John's, Nfld.,

TABLE 7. Mean and standard deviation of the log-transformed residuals (R) for equations 16 and 18, which describe the daily growth and mortality rates of postlarval fish in relation to temperature and average size, for different taxonomic groups of pelagic fishes. The standard deviations (SD) of all log-transformed residuals of equations 16 and 18 are 0.54 and 0.55, respectively.

Taxonomic group	Daily growth rates			Daily mortality rates		
	$\bar{R}$	SD	n	$\bar{R}$	SD	n
Clupeoids	0.03	0.35	27	−0.01	0.48	14
Gadoids	0.23	0.36	8	−0.20	—	1
Scombrids	0.48	0.52	7	0.53	0.41	6
Flatfish	−0.46	0.80	4	−0.48	0.25	2
Sebastes	−0.72	0.65	4	−0.32	0.60	4

TABLE 8. Summary of the effect of temperature and size on the daily development rate, the daily mortality rate, and the stage-specific cumulative mortality rate of fish eggs, yolk-sac larvae, and postlarvae. The effect of the variables is shown as the temperature and size parameters for the general model (eq. 4 or 6) that best described the data. Levels of significance: ns, not significant; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Note that the parameters for development rates of eggs and yolk-sac larvae are estimated using the formula $g[x] = 1/t_x$.

	Daily development rate		Daily mortality rate		Cumulative mortality rate	
Stage	T (°C)	Size (mm)	T (°C)	Size (mm)	T (°C)	Size (mm)
Eggs	0.099***	−0.44***	0.18***	ns	0.066**	ns
Yolk-sac larvae	0.071***	ns	ns	ns	−0.061*	ns
Postlarvae	0.073***	0.54**	0.067***	−0.68*	ns	−1.27***

pers. comm.; K. Drinkwater, Bedford Institute of Oceanography, Dartmouth, N.S., pers. comm.). Assuming a Q_{10} of 2.0, typical temperature anomalies could lead to a 32% variation in any given vital rate(s). Such large variations could lead to catastrophic fluctuations in survival (Houde 1987; Beyer 1989) unless there was strong compensation.

The results summarized in the previous section clearly indicate that the initial energy investment by adult fish into each offspring has very little influence on the cumulative stage-specific survival for the pelagic early life history stages of marine fish (eq. 10, 14, and 21). This is in contradiction with proposals based on life history theory (Stearns 1980, 1983) which suggest that the trade-off between offspring size and numbers is a reflection of the strategy of population growth potential versus stability in reproductive success, measured as the variability in mortality rates which should increase as the cumulative mortality increases. Several reviews have indicated that decreasing initial offspring size should lead to lower and more variable survival because of small energy reserves (Miller et al. 1988; Duarte and Alcaraz 1989), lower foraging ability (Miller et al. 1988), and higher susceptibility to predators (Pepin et al. 1987; Miller et al. 1988). Although the significance of the effect of initial size on overall cumulative stage-specific mortality rates appears to be negligible, there are size-dependent relationships for some of the components (i.e. daily rates) of the survival equation (cf. eq. 1).

The evidence suggests that increasing initial size reduces development rates during the egg stage, as found by Pauly and Pullin (1988) and Duarte and Alcaraz (1989), but that initial size has no significant effect on development rates of either yolk-sac or postlarval stages. Longer egg development times are required to produce larger larvae (eq. 11). The average size does have a significant effect on the growth rate of feeding larvae because larger/older larvae are more effective foragers (Hunter and Thomas 1974) and have relatively high search rates (Miller et al. 1988).

Daily mortality rates are independent of size during the nonfeeding stages (eggs and yolk-sac larvae) but decreases as the average size of feeding larvae increases. Stage-specific cumulative mortality rates showed a pattern similar to that of daily mortality rates. The lack of evidence for any size-dependent relationship in the daily mortality rates of the nonfeeding stages is in contrast with Peterson and Wroblewski's (1984) and McGurk's (1986) observations based on a comparison of data across all early life history stages. Peterson and Wroblewski (1984) matched their observations with a theoretical argument based on a continuous distribution of biomass across logarithmic intervals of size and the balance between production and predation. However, to properly compare results from this study with those of Peterson and Wroblewski (1984) and McGurk (1986), we must standardize equations 9 and 17, which describe daily mortality rates in relation to size and temperature for eggs and postlarvae, to 15.4°C, the mean temperature of observations for daily mortality rates of yolk-sac larvae (Table 5). Under these circumstances, the daily mortality rates of eggs would be 0.39, that of yolk-sac larvae would be 0.33, and that of 5-mm postlarvae would be 0.24, which is consistent with the observations of Peterson and Wroblewski (1984) and McGurk (1986) across developmental stages. Therefore, as development progresses, and consequently the size and/or shape of the animal changes, daily mortality rates decrease probably as a result of increasing ability to avoid predators. However, within individual early developmental stages, initial size appears to have little impact on daily mortality rates.

The strong size-dependent but temperature-independent relationship of stage-specific mortality rates of postlarval fish (eq. 21) is consistent with a reanalysis of Morse's (1989) data, which represent a completely independent source of information from that reported in Table 6. Data listed in Morse's (1989) table 5 (his Z_l, which is independent of estimated growth rates and equivalent to my C_L (Ebert 1973)) reveal that instantaneous length-specific (stage-specific) mortality rates are very significantly related to the average size of the larvae ($C_l = 6.36L^{-1.29}$, $r^2 = 0.67$, $p < 0.001$, $n = 26$). Residuals from this relationship are not significantly correlated with temperature ($r = 0.17$, $p > 0.2$, $n = 26$). This differs from Morse's (1989) interpretation. However, the size-dependent relationship is clearly stronger than the temperature-dependent relationship which he suggested is the dominant factor influencing stage-specific mortality rates of postlarval fish.

There is evidence in both in this and McGurk's (1986) study that an external factor influences the relationship between daily mortality rates and the size of the larvae. I propose that increases in mortality rates associated with increasing larval growth rates result from increased encounter rates with predators. High growth rates require high ingestion rates which in turn require that higher numbers of prey items be encountered. The higher activity required to increase ingestion would likely lead to increased encounter rates with predators (Gerritsen and Strickler 1977). McGurk (1986) suggested that increasing patchiness can result in increased mortality as a result of predation. Given that many features of marine ecosystems are correlated, deciding which hypothesis is accurate can only be done using comparative analyses, since experimental manipulations of whole systems are not possible. Alternatively, we may ask whether both theories are consistent with interannual variations in

growth, patchiness, and mortality within a species. Although repeated measurements of these variables are few, data for *Sebastes* spp. (Anderson 1984), *Clupea harengus pallasi* (McGurk 1987), and *Scomber scombrus* (Ware and Lambert 1985) are available. In all cases, mortality tends to increase with increasing growth rates whereas it decreases with increasing patchiness. Although this is not conclusive, the data appear to confirm that my findings based on differences between species are also applicable to differences between cohorts or year-classes of a species. It is possible that species which have high average mortality rates occur in physically dynamic environments. A study of McGurk's (1986) table 1 indicates that species which are patchily distributed occur principally in upwelling environments. These ecosystems are highly productive and hence should lead to high larval growth rates which are strongly correlated with high mortality rates.

Evidence from the averaged residual variance from the general temperature- and size-dependent models indicates that there are taxonomic adaptations in development and mortality rates beyond those described in terms of general models. Species that have long development times during the egg stage tend to have higher mortality rates, and species groups that have high growth rates also tend to have short yolk-sac stages. These findings appear consistent with previous concepts dealing with adaptive trade-offs (Pauly 1980; Roff 1984). However, there appears to be no association between group characteristics in the residual vital rates during the egg stage with group characteristics during the larval stages. It is possible that energetic trade-offs at one stage may be partly independent of trade-offs during other stages because of a wide range of likely life history adaptations (e.g. Roff 1984). However, the limited data set used in this study can only suggest that further research on this topic is needed.

It is clear from the analysis of postlarval daily growth and mortality rates that as size increases, the net mortality per unit growth decreases, possibly because bigger larvae are less susceptible to variations in environmental factors. Furthermore, variations in early life history survival are dependent on cumulative mortality which will ultimately be dependent on the change in length experienced during the larval stage as well as the time required by each individual within a population to reach metamorphosis. Although it is clear that we cannot study all the events which occur throughout the early life history, it is necessary to consider all the stages (i.e. egg, yolk-sac, and postlarvae) during which high mortality rates will be experienced rather than short developmental intervals in order to link variations in environmental conditions with survival and recruitment.

This study has characterized early life history development, mortality, and survival (cumulative mortality) in terms of basic temperature- and size-dependent relationships. Future efforts should be directed towards characterizing these vital rates in terms of the variability between individuals within a population (e.g. Chambers et al. 1988) or between stocks of a species (e.g. Blaxter and Hempel 1963). Once we can describe the effects of size or individual characteristics on vital rates, it may be possible to better understand the influence of other elements of the food web or of the physical environment on early life history survival.

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LETTERS AND COMMENTS
LETTRES ET COMMENTAIRES

Variation in Agonistic Behaviour between Hatchery and Wild Populations of Fish: A comment on Swain and Riddell (1990)

Swain and Riddell (1990) inferred from mirror-image stimulation experiments that juvenile coho salmon (*Oncorhynchus kisutch*) of hatchery origin showed higher levels of aggressiveness than wild strains. The authors cited the works of Fenderson et al. (1968), Fenderson and Carpenter (1971), and Moyle (1969) as empirical support for their general conclusion that hatchery salmon stocks are more aggressive. Fenderson et al. (1968) did indeed conclude that hatchery stocks of Atlantic salmon (*Salmo salar*) were more socially aggressive than wild stocks. In a later paper, however, Fenderson and Carpenter (1971, p. 446) modified their opinion and stated: ". . . the earlier conclusion that hatchery salmon are intrinsically more aggressive than wild salmon was misleading." Their new conclusion was that ". . . the comparative social aggressiveness of hatchery and wild salmon is density dependent, and that wild salmon are as aggressive. . .in low density as are hatchery salmon in high density." At any rate, the difference between Fenderson and Carpenter's "wild" and hatchery populations is purely environmental, since both are progeny of adults derived from natural spawning populations. This point was recognized by Swain and Riddell. However, what Swain and Riddell appear to have overlooked are the implications for their own results of the density-dependent factor in the expression of agonistic behaviour by both hatchery and "wild" populations (see below). Moyle (1969) reported that brook trout (*Salvelinus fontinalis*) of domestic origin performed more interactions between individuals than trout of wild origin, but he also explained this effect as the probable result of the higher swimming activity displayed by the domestic strain. Swain and Riddell did not discuss this important relationship between swimming activity and agonistic interactions, despite the fact that the point was stated by Fenderson and Carpenter (1971).

Swain and Riddell's own conclusions are based on mirror-image experiments. Mirror tests have the advantage of decoupling the confounding effect of size differential and dominance status because an individual fish interacts with its own mirror image (Metcalfe 1989). However, there are problems with the interpretation of such results, since mirror tests do not mimic several of the possible modes of interaction among individuals, even among those of the same size. Some of the categories of interactions allowed for in game theory are not represented in mirror tests (e.g. the assessor strategy, Maynard Smith 1982). A fish that escalates into a fight is always faced by a fish that also escalates. Conversely, a fish that swims away from a competitor (its mirror image) is always responded to by a fish that does the same thing. Using mirror tests, we do not know how an escalating fish reacts to a fleeing fish, or vice versa. Both behaviours are potentially relevant to the domestication process. The behaviour category under selection during domestication, according to Doyle and Talbot's (1986) game-theoretic analysis (cited by Swain and Riddell), involves behavioural switching as a function of relative size. These authors argued that "much of the phenotypic variation in agonistic behaviour does not come about because some animals are genetically more aggressive than others, but only because the animals which happen to be larger, for genetic or other reasons, adopt an aggressive behaviour mode" (Doyle and Talbot 1986, p. 1063). Since such behaviour is not observable during mirror-image stimulation, it is unclear why Swain and Riddell offer their results as a test of Doyle and Talbot's predictions. It is more difficult than Swain and Riddell suggest to extrapolate results obtained from mirror experiments to more natural situations in which pairs are allowed a broader range of alternative tactics in social encounters. The suggestion that Doyle and Talbot (1986) depreciated the importance of the correlation between aggressiveness and growth rate (Swain and Riddell 1990, final sentence) reflects a general misreading of the game-theoretic analysis of domestication. Doyle and Talbot's (1986, p. 1063) stated conclusion is that "the fish that grow fastest will be those that are uninvolved in competitive social interactions and can minimize the cost of aggressive and submissive behaviour."

An equally important, but more subtle issue is the problem of assessing the effect of a genotype–environment interaction. Swain and Riddell raised eggs of hatchery and wild origin in only one environment (the hatchery). From their experiments we do not know whether the fish of "wild" origin are inherently less aggressive than the hatchery strains, or whether it is the novel environment, for which the set of "wild" genotypes have not been adapted, that inhibits the expression of agonistic behaviour in these strains. Swain and Riddell's results indicate that we may expect "wild" fish to react less strongly than hatchery fish to conspecifics when they have been raised in a hatchery environment, with hatchery densities. But this says nothing about which fish might react more strongly in the wild at natural densities. Perhaps hatchery fish show lower overall activity levels in the wild than do wild fish. This argument, at least at the environmental level, is supported by Fenderson and Carpenter's (1971) finding that wild fish are more aggressive at low densities. Thus, in the absence of comparisons of juveniles of hatchery and wild origin raised both in a hatchery environment and in the wild (e.g. Barnett et al. 1979), any attempts to draw conclusions about what might happen if hatchery fish are released (e.g. Swain and Riddell 1990, p. 569) are unjustified speculation. An alternative approach to this 2 by 2 comparison (two sets of genotypes, two environments) is to carry out size selection experiments on fish of "wild" origin and to concurrently monitor the changes in agonistic behaviour that accompany the selection process.

There is concern among aquaculture geneticists that selection for rapid growth under competition would lead to higher levels of overall aggression, and therefore there would be no net gain in assimilation efficiency or growth (Kinghorn 1983). This concern is mitigated by the game-theoretic argument of Doyle and Talbot (1986) and by a large body of available evidence from

domesticated terrestrial and aquatic vertebrates (Hale 1969; Price 1984; Kohane and Parsons 1988). For example, laboratory-reared Norway rats (*Rattus norvegicus*) show a significantly lower intensity of aggressive behaviour than wild strains (Barnett and Stoddart 1969; Price 1978; Barnett et al. 1979). In addition, Holm and Fernö (1986) suggested a negative genetic correlation between growth and aggression in Atlantic salmon parr, and Robinson and Doyle (1990) found negative phenotypic correlations among aggressive behaviour and growth variables in tilapia (*Oreochromis mossambicus* × *hornorum*).

Swain and Riddell noted the important distinction between tameness and lack of intraspecific aggression. Certainly the weight of evidence favours the idea that tameness increases in fish under domestication as it does in other animals. The common carp (*Cyprinus carpio*) has been cultivated for more than 2000 yr and is extremely docile and tolerant of crowding (Weatherley 1976). Populations bred in captivity of Atlantic salmon, rainbow trout (*Oncorhynchus mykiss*), and brook trout appear to be tamer and less easily frightened than their wild counterparts (Vincent 1960; Holm and Fernö 1986; Gjedrem et al. 1987). Thus, there is increasing evidence suggesting that hatchery environments do indeed affect behavioural variables (both aggression and tameness) and that these changes may in fact be genetic. The possibility remains that the direction of these behavioural changes may be species specific or hatchery-regime specific. However, the emerging evidence seems to favour an increase in tolerance to conspecifics, not in aggressiveness — Daniel E. Ruzzante, *Biology Department, Dalhousie University, Halifax, N.S. B3H 4J1.* (JA711)

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Domestication and Agonistic Behaviour in Coho Salmon: Reply to Ruzzante

In our recent paper (Swain and Riddell 1990), we concluded that the available evidence indicated a genetic effect towards greater aggressiveness in hatchery than in wild strains of salmonids. We based this conclusion on experiments by Moyle (1969) with brook trout and on our own experiments with coho salmon. Moyle compared wild and domestic strains, both reared from fertilization in the same domestic environment. We compared recently emerged fry from hatchery and wild populations, reared from fertilization in the same environment and isolated at emergence both from other fry and from human disturbance. We noted that the results of these comparisons failed to support the prediction of Doyle and Talbot (1986) that selection in domestic environments should favour fish that are "uninvolved" in agonistic interaction. Ruzzante criticizes our conclusions and the use of our results to test this prediction. We believe that his criticisms misrepresent our procedures, our arguments, and the body of evidence bearing on the question of domestication and agonistic behaviour of salmonids.

First, Ruzzante states that we cite the works of Fenderson et al. (1968) and Fenderson and Carpenter (1971) as empirical support for our conclusions, and that we fail to consider the density-dependent nature of their results. In fact, we did note that the difference in aggressiveness between hatchery-reared and wild-caught parr was density dependent in their study (p. 567, paragraph 2, sentence 4). However, differences in their study were purely environmental, and Fenderson and Carpenter (1971) noted that their density effect may have reflected differences in the prior rearing conditions of their experimental subjects. On the other hand, our experimental subjects all experienced identical rearing conditions prior to testing, and our concern was for *genetic* divergence in aggressiveness between hatchery and wild stocks. Thus, we did not discuss the density dependence of their results in detail, nor did we cite their work as evidence that "artificial culture may lead to increased aggressiveness in salmon" (p. 570, last paragraph).

Ruzzante also criticizes our citation of Moyle (1969) as evidence for higher levels of aggressiveness in hatchery stocks, speculating that the higher rate of aggressive interaction among domestic fish in Moyle's study might result from their higher swimming activity. However, cause and effect are difficult to impute in this case. Moyle noted that "territories were much more clearly defined in the aquaria containing the domestic fry,

as the defending fish patrolled the territories vigorously." Thus, one might equally speculate just the opposite: that the greater activity of domestic fish resulted from their higher rate of agonistic interaction. At any rate, whatever the cause, the fact remains that domestic trout were more, not less, involved in agonistic interaction than were wild trout in Moyle's study.

Ruzzante questions our use of mirror experiments. He correctly notes that such experiments cannot incorporate certain modes of interaction, such as those between escalating and fleeing fish or those involving behavioural switching as a function of relative size. Because of these obvious limitations, he asserts that it is difficult "to extrapolate results obtained from mirror experiments to more natural situations." We agree that mirror experiments have limitations as models of interactions between individuals. However, our purpose was to test for differences in average behaviour between populations. At this between-population level, there is ample evidence, at least for coho and chinook salmon, that the results obtained from mirror experiments can indeed be extrapolated to more natural situations in which individuals may interact with a broad range of alternative tactics. This evidence was cited in our paper (p. 567, paragraph 2 under the heading "Mirror Image Stimulation"). Since publication of our paper, evidence has also accumulated that this conclusion holds for coho salmon even at the level of individual fish. Within populations, duration of agonistic behaviour in mirror experiments is significantly correlated with dominance ability in tests between paired individuals (following the method of Rosenau and McPhail (1987); $r = 0.70$, $N = 64$, $P = 0.001$, D. P. Swain and L. B. Holtby, unpubl. data) and with dominance ranking in stream tank tests among several individuals (following the method of Metcalfe et al. (1989); "swim-against-mirror": Spearman's $R = 0.54$, $N = 15$, $P < 0.05$, L. B. Holtby and M. Allen, unpubl. data). Thus, even though mirror tests do not mimic the full range of interactions possible among individuals in more natural situations, the evidence for coho and chinook salmon is that they are clearly a good predictor of agonistic behaviour in those situations.

Doyle and Talbot (1986) presented a game-theoretic analysis of selection for agonistic behaviour during domestication. Ruzzante claims that our results cannot be considered a test of their predictions because "the behaviour category under selection during domestication. . .involves behavioural switching as a function of relative size." While it is true that some of the phenotypes modelled by Doyle and Talbot exhibited size-contingent behaviour switching, the phenotype predicted to be favoured during domestication was not directly size contingent. It was not our intention to model the *process* of selection during domestication, but rather to test for differences in average phenotype between populations as a result of such selection. Doyle and Talbot predicted that growth rate selection in domestic environments will favour "fish which ignore each other" (p. 1059, Abstract) or, more precisely, uninvolved fish "which retreat the minimum necessary distance to avoid damage when attacked, otherwise ignore other animals" (p. 1061). In our experiment, hatchery fish were clearly *more*, not less, involved in agonistic interaction than were wild fish, a result opposite to the prediction of Doyle and Talbot.

Ruzzante states that we suggest "that Doyle and Talbot (1986) depreciated the importance of the correlation between aggressiveness and growth rate (Swain and Riddell 1990, final sentence)." We presume that he has misunderstood our final sentence. Our meaning was as follows. A concern of aquacultural geneticists is that selection for rapid growth may lead to higher levels of aggressiveness in domestic stocks because of a positive correlation between growth rate and aggressiveness (Kinghorn 1983). However, Doyle and Talbot predicted that growth rate selection in domestic environments will favour uninvolved fish, not aggressive fish, suggesting that this concern is not well founded. Ruzzante himself makes an equivalent statement (paragraph 4: "This concern is mitigated. . ."). On the other hand, our results indicate higher rates of agonistic behaviour in domestic than in wild stocks, suggesting that the concern may indeed be well founded.

Ruzzante raises the important issue of genotype–environment interaction. We know of no studies examining this issue with regard to agonistic behaviour of salmonids and agree that it is an area of research requiring attention. However, we do not believe that our results can be easily dismissed on the basis of genotype-environment interaction. Ruzzante invokes the results of Fenderson and Carpenter (1971). These results involved purely environmental effects on the same wild "genotype" (both wild- and hatchery-reared parr in their study were the progeny of wild parents) and so are irrelevant to the issue of genotype–environment interaction. Ruzzante claims that wild fish in our study were "raised in a hatchery environment, with hatchery densities," and suggests that the novel hatchery environment may have inhibited the expression of agonistic behaviour in these wild fish. As noted in our paper, neither wild nor hatchery fish were reared in "a hatchery environment, with hatchery densities" in our study. Instead, all fish were isolated at emergence in individual aquaria, an environment arguably as novel to hatchery as to wild fish. As noted above, agonistic behaviour in this novel environment is correlated among populations with behaviour in more natural environments, implying that genotype–environment interaction is not a problem in the interpretation of our results. Our results indicate a difference, probably genetic, in agonistic behaviour between wild and domestic strains in the environment tested. While we agree that it is important to investigate the possibility of genotype–environment interaction more fully, we feel that concern regarding the possible effects of agonistic interaction between hatchery and wild fish in the wild is prudent rather than "unjustified speculation" given these results.

Ruzzante concludes that the "concern among aquaculture geneticists that selection for rapid growth under competition would lead to higher levels of overall aggression. . .is mitigated by. . .a large body of evidence" and that there is increasing evidence that hatchery environments favour an increase in tolerance to conspecifics, not in aggressiveness. As support for these statements, he provides (1) some evidence of a negative correlation between growth and aggression in fish, (2) evidence that tameness increases in fish under domestication, and (3) evidence that laboratory-reared Norway rats are less aggressive than wild strains. Ruzzante's first point relates to the correlated response to growth rate selection to be expected during domestication. Only within-population correlations are relevant to this point. Correlations between growth and aggression among populations provide no information on expected responses to selection within populations. Of Ruzzante's two citations in support of this point, his only reference to the correlation in salmonids deals only with among-population correlations (Holm and Fernö 1986). Within populations, evidence on this first point is in fact equivocal, as studies not cited by Ruzzante indicate a positive correlation between growth and aggression in fishes (e.g. Yamagishi 1962; Metcalfe 1986; Knights 1987). Regarding the second point, we can only reiterate that a distinction must be

made between tameness and aggressiveness to conspecifics. The evidence is that domestic stocks of salmonids are less easily frightened by human disturbance than are wild fish (Vincent 1960; Gjedrem et al. 1987), but we know of no evidence for a negative correlation between this trait (i.e. "tameness") and aggressiveness to conspecifics. Finally, we believe that caution must be exercised when extrapolating results of behavioural studies from one vertebrate class (i.e. mammals) to another (i.e. fish). We conclude that there is very little evidence regarding effects of domestication on aggressiveness in fish and that all the direct evidence in salmonids suggests that aggressiveness is greater in hatchery than in wild strains (Moyle 1969; Swain and Riddell 1990).

When empirical tests fail to confirm theoretical predictions, it is reasonable to question the theory as well as its tests. Doyle and Talbot's (1986) model represents a novel approach to the analysis of effects of domestication on behaviour. However, it is unlikely that a single model of behaviour can be applied to all species and situations. For example, a critical assumption in Doyle and Talbot's analysis is that uninvolved fish conserve energy relative to involved fish by retreating when attacked instead of engaging in unnecessary display and submissive behaviour. This assumption may be reasonable in a population composed mostly of uninvolved fish. However, in an analysis of domestication and salmonid behaviour, the relevant question is whether an uninvolved phenotype can invade a population composed mostly of involved fish. We suggest that rare uninvolved fish would expend far more energy in frequent and possibly prolonged retreat than involved fish would in energy-saving displays (our observation is that failure to submit to a dominant fish can lead to a prolonged chase ending in one or more nips by the dominant individual). Involved fish may likewise lose few feeding opportunities due to agonistic interaction (e.g. Fernö and Holm 1986). In this case, selection for rapid growth would favour involved individuals, a prediction consistent with our empirical result and that of Moyle (1969).

In conclusion, we consider our work and that of Moyle (1969) to be only initial small steps in the empirical investigation of effects of domestication on agonistic behaviour of salmonid fishes. We studied only two pairs of hatchery and wild populations and noted that it was not possible to rule out the possibility that our results are explained by interpopulational differences unrelated to domestication. We believe that much more work needs to be done, including examination of more populations and species and investigation of factors such as genotype–environment interaction and density effects, as suggested by Ruzzante. However, decisions regarding selective breeding programs, the utilization of excess hatchery production, and the rebuilding of wild stocks are being made now. In making these decisions, we feel that it is imprudent to ignore the available evidence on effects of domestication on agonistic behaviour of salmonid fishes —

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ANNOUNCEMENTS/AVIS

Aquatic Toxicity Workshop

The 18th Annual Aquatic Toxicity Workshop will be held in Ottawa, Ontario, from 30 September to 3 October 1991. The theme of the workshop will be Ecological Perspectives on Aquatic Toxicology. Major topics will include (1) contaminant transfer through the abiotic and biotic compartments of the environment, (2) population and community responses to stresses, and (3) the role of aquatic toxicology in the regulatory agenda. A plenary session will be held on "Perspectives on aquatic toxicology: the government and research communities."

A special Arctic session "Contaminants in Arctic Ecosystems" will include speakers from Sweden, Norway, Denmark, USSR, the United States, and Canada.

Platform and poster sessions will be accepted in English or French (there will be simultaneous translation) and abstracts must be received by 2 July 1991 to be considered. For further information: Dr. Margaret C. Taylor, Water Quality Branch, Environment Canada, Ottawa, Ontario K1A 0H3, Canada. Telephone (819) 953-1553; FAX (819) 997-8701.

Atelier sur la toxicité aquatique

Le dix-huitième atelier annuel sur la toxicité aquatique se tiendra à Ottawa (Ontario) du 30 septembre au 3 octobre 1991 et aura pour thème les perspectives écologiques sur la toxicité aquatique. Parmi les principaux sujets qui y seront abordés, citons : 1) transfert des contaminants par les composantes biotiques et abiotiques de l'environnement, 2) les réponses des populations et des communautés aux stress et 3) le rôle de la toxicologie aquatique dans le plan d'action sur la réglementation. Une séance plénière concernant les vues des fonctionnaires et des chercheurs sur la toxicologie aquatique sera aussi au programme.

Au cours d'une séance spéciale portant sur l'Arctique et intitulée « Les contaminants dans les écosystèmes arctiques », on pourra entendre des conférenciers en provenance de la Suède, de la Norvège, du Danemark, de l'URSS, des États-Unis et du Canada.

La Direction acceptera jusqu'au 2 juillet 1991 les résumés des communications en anglais ou en français pour les séances thématiques et celles d'affichage (un service d'interprétation simultanée sera disponible). Pour obtenir de plus amples renseignements à ce sujet, prière de communiquer avec Mme Margaret Taylor, Direction de la qualité des eaux, Environnement Canada, Ottawa (Ontario) K1A 0H3, Canada. Téléphone (819) 953-1553; télécopieur (819) 997-8701.

Call for nominations

ECOLOGY INSTITUTE PRIZES 1991

in the field of Limnetic Ecology

Call for nominations

Financed by research ecologists and non-profit-making, the international Ecology Institute (ECI) has a staff of 41 ecologists – marine, terrestrial and limnetic – all of high professional reputation. Neither the director nor any member of the scientific staff receives remuneration.

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Taking into account these aims, as well as his or her own field of interest, the winner of the Ecology Institute Prize is requested to author a 200 to 300 printed-page book, to be published by ECI in the series 'Excellence in Ecology' (EE) and to be made available worldwide at cost price. A considerable number of books are donated to libraries in Developing Countries.

EE's concept is different from that of textbooks. In addition to reviewing a certain field of knowledge, it gives the authors a chance to express their personal views on important ecological issues, to interpret current scientific knowledge on the basis of their own experience and insight, and to tell us what, in their opinion, should be done in the future.

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Providing adequate nominations are received, a second prize may be awarded honoring a young ecologist who has conducted and published results of uniquely independent, original and/or challenging research representing an important scientific breakthrough: the IRPE PRIZE (International Recognition of Professional Excellence) is endowed with a stipend of US $ 750.

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1986 Marine Ecology:	Tom Fenchel, Helsingør, Denmark – ECI PRIZE. Book 1: 'Ecology – Potentials and Limitations' (Published 1987; price DM 67 plus DM 5 for postage and handling)
	Colleen Cavanaugh, Cambridge, MA, USA – IRPE PRIZE
1987 Terrestrial Ecology:	Edward O. Wilson, Cambridge, MA, USA – ECI PRIZE. Book 2: 'Success and Dominance in Ecosystems: The Case of the Social Insects' (Published 1990; price DM 67 plus DM 5 for postage and handling)
1988 Limnetic Ecology:	Gene E. Likens, Millbrook, NY, USA – ECI PRIZE. Book 3: 'Insights from Sustained Ecological Research: An Ecosystem Perspective' (The book is scheduled to appear in 1990)
1989 Marine Ecology:	Robert T. Paine, Seattle, WA, USA – ECI PRIZE. Book 4: 'Ecological Pattern and Process on Rocky Shores' (Tentative title)

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